SPECIAL EDITION FOR IGEM STUDENTS

Genetically Modified Organisms

Environmental Risk Assessment Guide

2nd Edition

iGEM

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ILSI BRASIL

INTERNATIONAL LIFE SCIENCES INSTITUTE DO BRASIL

Rua Hungria, 664 - conj.113

01455-904 - São Paulo - SP - Brazil

Tel./Fax: 55 (11) 3035 5585 e-mail: ilsibr@ilsi.org.br

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Cover photo: A field trial of GM maize in Central America. Photo by WA Parrott, 2012

Genetically Modified Organisms – Environmental Risk Assessment Guide

Editors

María Mercedes Roca, **Professor, Department of Biotechnology and Bioengineering, Tecnológico de Monterrey, Guadalajara Campus, Mexico, mmroca@itesm.mx**

Wayne Parrott

Professor, Department of Crop and Soil Science, University of Georgia, United States. wparrott@uga.edu

Paulo Paes de Andrade

Professor, Department of Genetics, Federal University of Pernambuco, Brazil. andrade@ufpe.br

English Version

Robert McDowell, Senior Economist, Consultant MSR, rmmcd_99@yahoo.com. Consider including his full title Risk Analyst / Senior Economist USDA- APHIS (retired)

Monica Garcia-Alonso, mgarcia@estelconsult.com, Estel Consult Ltd, Independent consultant, UK

Authors & contributors

Ederson Akio Kido

Professor, Department of Genetics , Federal University of Pernambuco, Brazil

Elizabeth Hodson de Jaramillo, Emeritus
Professor, School of Sciences, Pontificia Universidad Javeriana, Colombia

Fernando Carlos Zelaschi

Biosafety Area, Biotechnology Division, Ministry of Agriculture, Livestock and Fisheries, Argentina. Responsible for the Biosafety Evaluation Unit, Biotechnology Division. Secretariat of Agriculture, Livestock and Fisheries, Argentina

Francisco José Lima Aragão

Senior Researcher, Embrapa Genetic Resources and Biotechnology, Brazil.

Gutemberg Delfino de Sousa

Principal Professor, Faculty Anhanguera de Brasília, Brazil; Advisor to the Biosafety National Technical Commission, Brazil

Isabel Saad Villegas

Researcher, National Autonomous University of Mexico (UNAM)

Jose Luis Solleiro Rebolledo

Researcher of Innovation and Management Policies, Center for Applied and Technological Development, Autonomous National University of Mexico, Mexico.

Josias Corrêa de Faria

Researcher at Embrapa Rice and Beans, Brazil

Marcia Almeida de Melo,

Professor Center for Rural Health and Technology, Federal University o *Campina Grande*, Brazil.

María Mercedes Roca

Professor, Department of Biotechnology and Bioengineering, Tecnológico de Monterrey, Guadalajara Campus, Mexico

Moisés Burachik

Director of Regulatory Affairs at the Institute of Agro-Biotechnology Rosario (INDEAR), Argentina

Paulo Paes de Andrade

Professor of the Department of Genetics, Federal University of Pernambuco, Brazil.

Sol Ortiz García

Executive Secretary. Executive Secretariat of the Intersecretarial Commission for Biosafety and Genetically Modified Organism (CIBIOGEM), Mexico.

Wayne Parrott

Professor, Department of Crop and Soil Science, University of Georgia, United States

DISCLAIMER

The contributions are solely those of the authors based on their technical and professional expertise, and do not necessarily represent the views or policies of their employing organizations.

This guide is still under revision. It is the intention of the editors to publish the guide in this unfinished version so iGEM students can get a sense of what an Environmental Risk Assessment (ERA) of a Genetically Modified Organism (GMO) involves. This is not the final version of the second English edition of the previously published ERA guide (2012).

Many consider synthetic biology an evolution of biotechnology. Thus, the same principles of an ERA for a GMO may apply to organisms and products of synthetic biology. A case-by –case approach is always advised when conducting an ERA. This is especially true for ERAs applied to synthetic biology.

This special edition was prepared for iGEM students by Maria Mercedes Roca, Tecnológico de Monterrey – Campus Guadalajara, Mexico. September 2015.

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Preface

Modern biotechnology has numerous applications in medicine, industry, environment, energy and agriculture, where it has contributed significantly to agricultural production, food security, and food safety. It has the potential to improve sustainability in a variety of ways by maintaining natural ecosystems as well as agricultural biodiversity.

In the last decade, the merging fields of biotechnology, nanotechnology, engineering, information technology and related fields have merged into an "evolution of biotechnology" that many call synthetic biology or systems biology. This emerging field of biology aims at designing and building novel biological systems, and potential risks, as with any technology, need to be evaluated and regulated.

Modern biotechnology has been used to introduce genetic modifications into organisms, and these organisms are known as transgenic or Genetically Modified Organisms (GMOs). The Cartagena Protocol on Biosafety of Biotechnology to the Convention on Biological Diversity refers to these organisms as "Living Modified Organisms" (LMOs), i.e., genetically modified organisms with the ability to reproduce. For the purposes of this guide, a GMO is any living organism, including plants, animals and microorganisms, containing added or altered genetic material by applying recombinant DNA (rDNA), gene silencing or genome editing techniques.

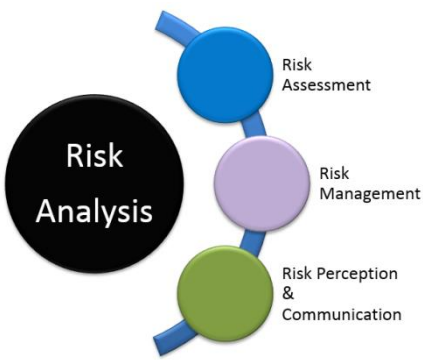


Figure 6: The three components of risk analysis. The decision whether or not to liberate a GMO is based on the information that comes from the Risk Analysis.

GMOs represent valuable products and tools in medicine, industry, the environment and agriculture that can bring a wide range of benefits; thus their development continues to get more sophisticated and precise and their use continues to grow globally. Despite the enormous potential benefits of GMOs, legitimate concerns relating to potential risks they may pose to the environment or to human or animal health remain. As a response, GMOs are strictly regulated and each product is scientifically assessed, taking into account the characteristics of the product, including the new trait, the intended use and the environment where it will be released or confined.

Prior to a regulatory decision, a risk analysis is undertaken. The ensuing decisions can range from approval, conditional approval, a request for more data, to denial.

Risk analysis includes three components as seen in Figure 1. Risk Analysis is applied to many fields involving regulatory decision-making and is not unique to GMOs.

A key part of any risk analysis is risk assessment. Traditional risk assessment methods are used for the Environmental Risk Assessment (ERA) of GMOs, having been adapted to the specific aspects of these types of organisms. One of the key issues surrounding ERAs for GMOs is that there is no international consensus on a single/standardized approach and different countries and regions follow varying approaches. Some countries have detailed data requirements that must be fulfilled in order to complete the ERA, while others not only prescribe what data has to be generated, but also which methods have to be used to generate this data. In some regions the procedure by which the GMO was developed is evaluated, while in others, only the risk posed by the final product is evaluated, regardless of the method used to develop it. The result is an array of different approaches to data collection and interpretation that generate confusion and lead to duplication at best, or inaction for approval at worst.

The main objective of this document is to provide flexible, practical guidance on the key principles of a risk assessment methodology to use for assessing potential environmental risks that may arise from developing a GMO for confined used, such as the recombinant microorganisms used in industrial biotechnology (bioreactors), or introducing GM crops or other GMOs into the environment. In the latter scenario, where the GMO is released into the environment, the risk assessment poses more challenges due to uncertainty than in the case of GMOs produced for confined use.

The first edition of this guide published in 2012 and directed at the ERA of genetically modified crops, represented a collective effort by Latin American biotechnologists and regulators to present a practical and efficient process for ERA. The second edition has been translated into English. It describes the principles of the discipline of risk analysis that can be applied to any current GMO, developed by modern biotechnology or synthetic biology for confined use or for release into the environment. The authors fully acknowledge the need to take a case-by-case approach when evaluating the risks of each individual GMO, and recognize the need to adapt and develop new ERA methodologies as new technologies develop.

This guide is divided into two sections: Part I focuses on the principles of ERAs that are carried out using a scientific process, having the step of “problem formulation” at its core and evaluating the final product, not the process. Part II includes a specific case study of an ERA with step-by-step instructions, demonstrating the science-based process of risk assessment used in many countries in Latin America where GM crops are developed and used commercially.

This second edition in English, still in draft form, is equally useful to non-Latin American countries that are signatories to the Convention of Biological Diversity and the Cartagena Protocol. Finally the guide also aims to help iGEM (International Genetic Engineering Machine) teams learn the basic principles of how to conduct an ERA to evaluate the risk of their recombinant organism (a GMO) developed through synthetic biology.

We emphasize that the recommendations in this guide meet the recommendations for risk assessment outlined in Article 15 and Annex III of the Cartagena Protocol on Biosafety, which are appropriately flexible to meet the individual needs of iGEM teams and ultimately, their countries.

Guide for Environmental Risk Assessment

PART I: The conceptual basis of Risk Assessment of genetically Modified Organisms (GMOs)

Chapter 1: Introduction

1.1 Biotechnology is evolving into synthetic biology

The fields of application of biotechnology, nanotechnology, engineering and information technology are merging into synthetic biology.

It is well recognized that human activities throughout history have had a significant impact on the environment. Among these activities, industrialization, synthetic chemical processes, an oil (petroleum) – based industry and especially the adoption of agriculture to ensure a regular supply of feed, fiber, food for increasing populations has contributed to climate change, deforestation, species extinction, soil erosion, air and water pollution, and harm to other natural resources. The last century closed with a clear understanding that natural resources are limited and must be preserved: society now recognizes that the sustainable use of natural resources is essential for humanity's survival, especially as the population is expected to exceed nine billion in the coming decades. For these reasons, new technologies are being developed to reduce the impacts caused by many human activities in the past and ensure the sustainability of the planet. As the threats of climate change loom larger, there is a growing recognition by society that we need to move from an fossil fuel and chemical - based economy, to a bio-economy based on the development of environmentally friendly and more efficient processes.

The exponential growth of biotechnology techniques and the powerful and inexorable convergence and fusion of biotechnology, nanotechnology, and related technologies have opened the door to novel, more sophisticated and precise tools and approaches. These tools can either significantly contribute to meeting the many challenges faced in the 21st century, or be used by bioterrorist for nefarious purposes to harm humans and the environment. What we do with these technologies is not pre-ordained, nor has the future been written. It is, however, imperative that the developers and users of these powerful technologies take a scientific-based approach to assess the potential risks.

At this stage (2015) the authors purposely stay away from offering an operational definition for synthetic biology, as many such definitions have been put forward by different groups, reflecting their background. In these early days of synthetic biology, the authors offer a set of basic principles within the discipline of Risk Analysis that can be applied to the ERA of any current GMOs. Through the case study included in the guide, we present a step-by step approach used to assess a GM crop in Latin America.

1.2 History of GMO regulation and biosafety systems for biotechnology

The commercial use of GMOs began in the last decades of the 20th century. The first products were developed in the 1970s and 80s for medical and food use, using GM microorganisms grown under containment to produce pharmaceuticals (e.g., human insulin) or food processing aids (e.g., chymosin for cheese). In the 1990s the technology was applied for agricultural and industrial uses.

When recombinant DNA technology was first developed in the 1970s, the perception was that scientists could now produce versions of organisms that would not normally be found in nature, as genetic material could be transferred between unrelated species. This generated concerns regarding the potential risks from GMOs to human health and the environment, including potential adverse consequences for biodiversity.

In 1975 the *Asilomar Conference on Recombinant DNA,* was organized by a group of scientists in California, to discuss the potential biohazards and regulation of biotechnology. A group of about 140 professionals (primarily biologists, but also including lawyers and physicians) participated in the conference to draw up voluntary guidelines to ensure the safety of recombinant DNA technology. The conference also placed scientific research further into the public domain. In 1992 the Conference on Environment and Development (also known as the "Earth Summit") was convened by United Nations in Rio de Janeiro, Brazil. 172 governments participated, with 116 sending their heads of state or government. Some 2,400 representatives of non-governmental organizations (NGOs) attended, with 17,000 people at the parallel NGO "Global Forum".

An important achievement of this influential summit was an agreement on the Convention of Climate Change, leading to the Kyoto Protocol and the Convention on Biological Diversity (CBD), which in turn led to the Cartagena Protocol on Biosafety of Biotechnology (CPBB). The Cartagena Protocol was signed and later ratified in 2003

by most member states (Parties) of United Nations and established the international biosafety framework, on which signatory member states were required to base their biosafety laws and regulatory policies concerning biotechnology. Notable exceptions to ratifying the Cartagena Protocol (to this date), include the USA, Canada, Argentina and Australia - all important agro-exporting countries.

Another important outcome of the Earth Summit, was **"Agenda 21"**, an action plan for sustainable development for the 21st century, presented as a 700 page document divided into 40 chapters, grouped in 4 sections:

- **Section I**: *Social and Economic Dimensions*, directed towards combating poverty, especially in developing countries, changing consumption patterns, promoting health, achieving a more sustainable population and sustainable settlement in decision-making.
- **Section II**: *Conservation and Management of Resources for Development,* including atmospheric protection, combating deforestation, protecting fragile environments, conservation of biological diversity (biodiversity), control of pollution and management of **biotechnology** and radioactive waste.
- **Section III:** *Strengthening the Role of Major Groups*, including the role of children and youth, women, NGOs, local authorities, business and industry, and workers; and strengthening the roles of indigenous peoples, their communities and farmers.
- **Section IV**: *Means of implementation,* including science, technology transfer, education, international institutions and financial mechanisms.

The Cartagena Biosafety Protocol requires Parties to make decisions on "import of Living Modified Organisms (LMOs) for intentional introduction to the environment based on scientifically sound risk assessment". Anex III of the Protocol states general principles, methodological steps, and points to consider in the conduct of risk assessment. The general principles include, among others, the following concepts: risk assessment should be carried out in a scientifically sound and transparent manner; lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, and absence of risk, or an acceptable risk; risk should be considered in the context of risk posed by the non-modified recipients or parental organisms; and that risk should be assessed on a case-by-case basis.

In the area of agricultural technology, more than two decades after the first introduction, concerns still exists regarding the coexistence of GM crops with conventional varieties, especially those used in organic production systems, and with sexually compatible wild relatives, in centers of origin or diversification. The safety

considerations related to gene technology in agricultural crops can be broadly grouped into two aspects: food and feed safety (toxicity and allergenicity) and environmental safety (such as invasiveness, harm to non-target organisms and harm to biodiversity).

To address these concerns, safety measures were put in place and biosafety laws and mechanisms were established in most countries. In the early days of genetic engineering, these safety measures focused on applying containment measures and safe working practices in laboratories to prevent the release of experimental material for which the risk to humans or animals was unknown, and to protect the scientists working with those materials from possible harm. With the development of GM crops that were intended for release into the environment, the concept of biosafety evolved. Biosafety is now understood as a combination of policies, rules, and procedures to ensure an adequate level of environmental and human protection from the use of GMOs.

The main objectives of biosafety systems are to prevent, manage, mitigate, minimize, or eliminate health and environmental harm that may result from releasing biological agents into the environment, and to protect the environment from biological agents and organisms used in research and trade. Biosafety in its general context includes legal, scientific, technical, administrative and institutional components that manage any associated risks to ensure they remain at an acceptable level.

The principles of biosafety are applied to all GMOs, meaning that GM crops have been strictly regulated since their initial development.

Risk assessments are conducted to evaluate any potential adverse effects to human and animal health and to the environment. Risk assessment is based on scientifically sound and transparent procedures. For those GM crops that may be used as food or feed, or used to produce food and feed products, there is a universal concern to ensure human and animal safety. Therefore potential adverse effects on human health, such as allergenicity and toxicity, are carefully evaluated. The methodologies used for the risk assessment of food and feed are well developed, recognized as robust, and fairly harmonized internationally. This is mainly due to the fact that effects on humans do not vary substantially among populations. Differences in preparation and level of consumption of given foods in different countries are taken into account during the risk assessment.

In contrast, the methodology for ERAs is not yet well harmonized. The perception is that the environmental characteristics of each region are unique, and the species in each region that may be at risk are very varied. Thus different countries may have different approaches focusing on different aspects of the environment that they wish to protect. While some countries rely heavily on agricultural production and seek a balance between agriculture and biodiversity, other countries put a higher emphasis

on protection of natural environments and see industrial agriculture and the use of GM crops as a threat. On way to stop or stall introduction of GMOs into a given environment (country) is through stringent regulation. As a result, several approaches to risk assessment of GMOs have been developed with varying degrees of “precaution”.

1.3 Conventional and modern plant breeding techniques

For centuries, conventional breeding techniques have been used for the domestication and improvement of crops, with the aim to provide better yields and food quality. Charles Darwin pointed out in *The Origin of the Species* that the process of domestication has led to crops that bear little resemblance to the wild plant species from which they were originated. Crop modification was initially achieved by selecting more desirable phenotypes and crossbreeding between varieties. In the last century, scientific methodology incorporating the laws of Mendelian inheritance was applied to this process, giving birth to what is now known as modern plant breeding. In the last 3 decades biotechnology and genetic engineering were incorporated into plant breeding and more recently, more precise and powerful technologies like RNA interference (RNAi) and genome editing through CRISPER-CAS9 and other related technologies, have been added to the arsenal of available tools.

Modern biotechnology allows the transfer or modification of genetic material among organisms by using recombinant DNA techniques (rDNA) to incorporate new traits into crop plants, which would have been either impossible or extremely difficult to incorporate or isolate with conventional methods. There are many parallels between the conventional production of new crop varieties and the production of a commercial Genetically Modified (GM) crop, shown in Figure 2.

The starting point in conventional breeding is frequently a trait of interest. This trait can come from a wild relative, from another species that can be intercrossed, albeit with difficulty, or even from an induced mutation. The advent of genetic engineering added to the possible sources of new traits. The plant with the trait of interest is then crossed with another parent, and the conventional breeding process starts, during which only plants with the desired characteristics are selected at each stage; plants that do not satisfy the quality requirements are discarded, resulting in a single plant that will form a variety that will be commercially acceptable. As a result, crop varieties are continuously evolving and new diversity is created in the process.

For GM, the trait can come, in theory, from any organism. A large number of events are screened to find a modified plant , known as the lead event, in which the introduced gene meets several quality criteria and the trait behaves exactly as intended. For crops normally planted from seed, one or more lead events are then used as parents which are bred together, at which point it starts going through the conventional breeding process before it is commercialized. Thus, breeding and transgenics are complementary technologies; one is not a replacement for the other.

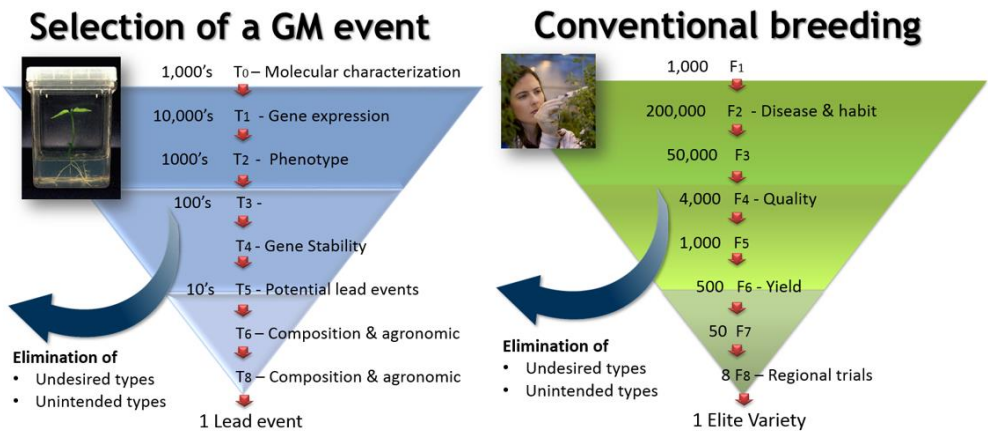


Figure 7: Comparison between conventional breeding and the development of a commercial GM crop variety. In both cases the process begins with a large number of plants that are progressively reduced according to their performance in several rigorous screens. Due to this stringent selection process, uncertainties about safety and agronomic performance are reduced accordingly, making it highly unlikely that undesirable varieties will be marketed. The lead event will still go through a conventional breeding scheme before reaching the market. The conventional breeding scheme is adapted from http://www.generationcp.org/plantbreeding/index.php?id=052.

What is an ‘event’?

The word 'event' is often used when speaking of a GMO. An event is simply a GMO that has been derived from a single transgenic cell. Thus each event is a GMO that was created independently. Whereas GMOs can contain the same gene, each time an event is created, the transgene will in all likelihood insert in a different position of the genome. Thus, using current technology, there are no two independently derived GMOs that have the transgene in precisely the same position of the genome. Because the position in the genome can affect gene expression, each independently derived GMO may have slightly different characteristics. For this reason, each independently derived GMO is called an **event**. Usually hundreds or thousands of events are created, of which only one (or a few) is selected for commercial purposes, as shown in Figure 2.

Chapter 2 : Risk Analysis of Genetically Modified Organisms

2.1 Risk analysis and risk assessment in the context of this guide

Risk analysis in the context of ERA is the systematic use of information to guide decision-making on the basis of risks and benefits of a particular technology or product. Risk analysis is an integrated process that consists of three components (Rudloff, 2004):

- **Risk Assessment** is a rigorous science-based process used to estimate the probability and impact from human activities on something that is being protected. The process involves, in a comparative basis, identifying hazards, characterizing those hazards and estimating potential exposure to them, to obtain qualitative or quantitative measures of risk.

- **Risk Management** is based on the risk assessment considering the country's protection goals and evaluates the new risks that are identified, their acceptability, and potential mitigation measures. It takes into account the potential benefits. If necessary, it defines and implements the most appropriate control measures and includes control and monitoring mechanisms. Even when levels of risk are found to be high, if the benefits outweight the risk, it is feasible to implement risk management measures to prevent, mitigate or control those risks to acceptable levels. The appropriate risk management scheme depends on the cost of mitigation, the resulting reduction in risk, and resulting negative consequences and positive benefits.

- **Risk Communication** is the interactive exchange of information among various stakeholders on potential risks and benefits and their management in such a way that informed decisions can be made. It involves addressing real and perceived risks using an open dialogue among regulators, technology developers, decision makers, and the public.

Risk assessment considers only those hazards (i.e., possible adverse effects) that are **biologically plausible** *and* **lend themselves to scientific assessment** using empirical and other validated methods to test hypotheses. It is based on formulating biologically plausible hypotheses about risk, rather than advancing speculative possibilities, which mainly reflect curiosity or personal concerns.

Hazard and risk in the context of Risk Assessment

The difference between risk and hazard is a fundamental concept in risk analysis. A very general definition of risk is the probability of harm that would result if a particular event (i.e., the hazard) were to take place, as illustrated in Figure 3:

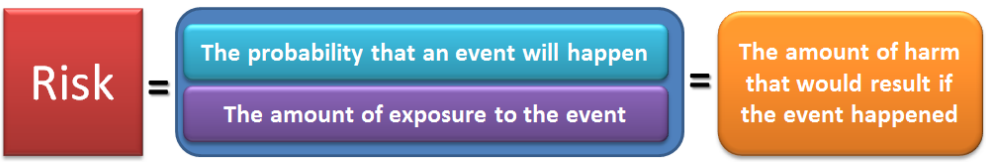


Figure 8: The components that determine risk. Different societies and countries differ in their tolerance to different types of harm, and hence perceive a given risk differently.

Hazard then, is simply something with the *potential* to cause harm. It follows from Figure 3 that risk assessment must first determine which are the main concerns (hazards), then determine how likely it is that that each of these could happen, and then determine the amount of possible harm that could result.

Harm depends on the significance of the effect and its likelihood of occurrence (exposure). However, because "harm" is a subjective concept, risk has different meanings depending on the social, cultural and economic characteristics of a given society. *It is therefore important to focus the risk assessment on biologically plausible factors that can be objectively measured.*

During this process, it is important to remember that no human activity, however simple, presents zero risk. Frequently lack of activity or inaction (i.e., choosing not to adopt new technologies, such as chlorination of public water supplies, immunizations, etc.) may involve greater risk than adopting a new technology, despite all perceptions to the contrary.

There are several possible effects that the release of any organism might have on an environment, regardless of whether they are conventional (e.g. biological control agents), modified by conventional methods (e.g irradiated insects) or by modern biotechnology (e.g GM crops). In the case of GMOs, the aim of risk analysis in most jurisdictions, and more specifically of risk assessment (as part of risk analysis), is to determine if GMOs could pose risks *different* from those presented by the conventional or non-GM organism (or already approved GMOs) currently in use that could lead to more harm to the environment. For this reason, a comparative approach is followed, where the GMO and its non-GM counterpart are always compared in the same context.

Based on this fundamental premise, risk analysis is carried out in three phases: risk assessment, risk management and risk communication. Risk perception is commonly considered as part of risk communication. Collectively, these components are the foundation from which decision-makers can decide whether environmental release of a GMO can take place or not. Figure 4 shows the relationship among these components.

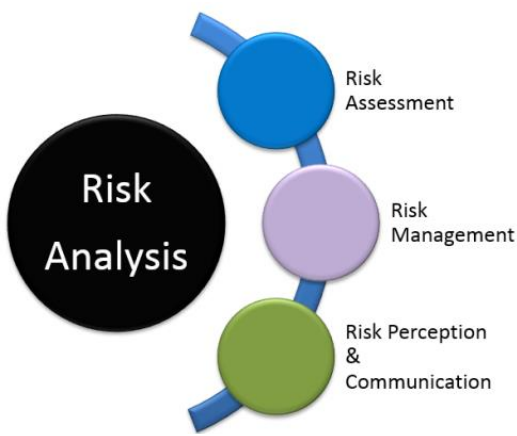


Figure 9: Risk analysis and its main components: including risk assessment, management and communication (adapted from Wolt et al., 2010), leading to a decision. The risk assessment can be revisited based on new information after commercialization, and gathered as part of risk management or communication. The two figures show that final decisions can range from purely scientific, to purely political, depending on the weight given to nonscientific issues.

Note from Figure 4 that risk assessors are generally not the ones who make a decision about whether to permit commercial planting. Risk assessors (technically trained people with the right scientific credentials) ONLY ask and answer one question: Is it likely that the GMO could harm food or feed safety, or the environment in a novel way? The assessment provides an estimate of likelihood, but does not make decisions of any kind. Risk analysis needs to then determine if the risks identified by the risk assessment are acceptable, or if they can be managed in order to mitigate or avoid them. However, the acceptability criteria can vary from country to country, and it is a policy-based decision.

Officials in charge of making decisions can then use the information from risk assessors to determine the final course of action. For commercial releases (as opposed to confined field plots), social and economic issues are additional considerations, which while they are not part of the risk evaluation itself, can affect decision-making in some jurisdictions. Evaluation of such issues is a task for experts on economic and social issues, and not environmental risk assessors, as it requires a different set of skills and knowledge to those commonly held by risk assessors.

Since risk or mitigation measures involve both economic and environmental costs, the environmental benefits of the use of GMOs should be evaluated and compared with possible harm to determine if commercial authorization is favorable, or, on the contrary, should be denied (Figure 11). For example, in the case of GM crops, economic (Wesseler et al, 2011) and environmental benefits from the reduced use of insecticides and from soil conservation from reduced or no-tillage are well documented (Carpenter 2010). The reduction of some types of mycotoxins is an additional beneficial effect in Bt maize (Wu, 2006).

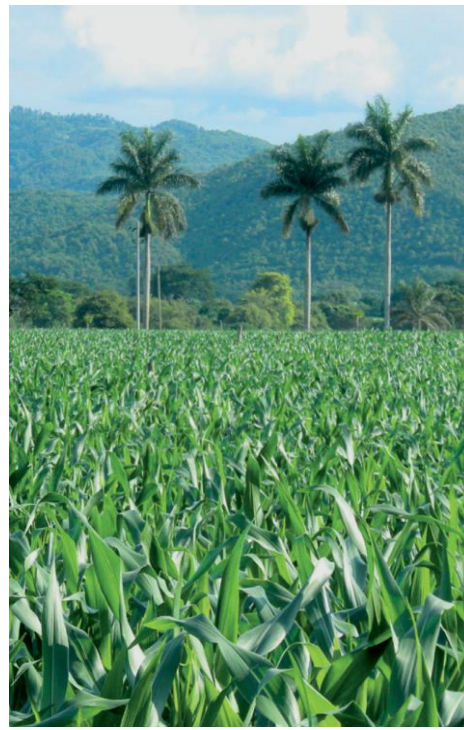
2.2 Cartagena Protocol and socio-economic considerations

Article 26 of the Cartagena Protocol provides that Parties “may take socio-economic considerations into account in accordance with their international obligations." The language of Article 26 specifically uses the phrase ‘may take into account’ - which means that each Party may use their discretion on whether or not to take socio-economic considerations into account. Parties are in full compliance with the Protocol when they exercise their power to exclude socio-economic considerations from risk analysis.

In addition, Parties must act “in accordance with their international obligations”. This language is a reference to the obligations of the Parties that are also members of treaties governing world trade, more specifically of the Agreement on the Application of Sanitary and Phytosanitary (SPS) Measures. In the framework of the SPS Agreement, the Parties commit to use scientific principles and science-based evidence to make decisions in risk assessment.

In the last three paragraphs of the preamble of the Protocol, the Parties agree to support each other in trade and in international environmental agreements. The Parties effectively meet this mutual support through the exercise of their discretionary powers (under Article 26) to exclude socio-economic considerations in risk analysis. In fact, taking into account socio-economic considerations could create conflict between international trade agreements and the environment, since they are not based on scientific principles or evidence-based science.

Chapter 3: Environmental Risk Assessment (ERA)



It is important to differentiate the steps that are followed in risk assessment from other aspects of risk analysis, which are presented in detail Chapter 2. The risk assessment of GM crops is conducted in a comparative manner. Since conventional crops have been grown for centuries, their agronomic properties are well known, and they have been used safely in food and feed. This is the basis of the concepts of "familiarity" and "history of safe use" developed in international consensus documents such a Codex and OECD (Codex, 2009; OECD, 1993a; 1993b).

Therefore this known behavior is used as a baseline, and the risk assessment can focus on identifying the meaningful differences that have resulted in the GM crop as a consequence of the genetic modification, and that can lead to harm. The general idea is to compare the GM crop with a suitable comparator (usually the conventional crop or a variety with a history of safe use) and determine if the genetic modification has led to potentially harmful differences (hazard identification). These differences can either be "intended" (i.e., the intended effect of the genetic modification, such as herbicide tolerance or insect resistance) or "unintended" (i.e., expected or unexpected differences that appear as a consequence of the genetic modification, such as increased weediness).

Once the phenotypic differences between the comparator and the GM version have been identified, the risk assessment focuses on those

differences with the potential to cause harm (potential hazards) as well as the likelihood and the magnitude of the possible adverse effects, to come up with an estimate of risk.

3.1 Problem formulation in Environmental Risk Assessment

For GM crops, the nature of the hazards and the pathways of exposure can be diverse. Thus, risk assessments are conducted on a case-by-case basis, and take into account the crop (or any other GMO), the gene(s) inserted, the traits expressed, and the environment where the crop will be grown. In many cases quantitative measures of risk are not possible and qualitative measures are used.

Given this case-by-case approach, the very first task in any risk assessment for GM crops is the "problem formulation," followed by problem characterization. It involves planning, information collection and selection of assessment endpoints. While every problem formulation must cover the same components, there is flexibility in the way these components are addressed (Figure 5).

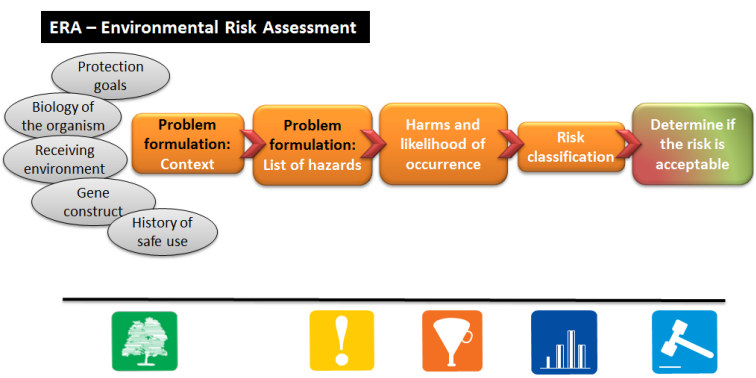


Figure 10: The different parts of environmental risk assessment, beginning with the factors that lead to the identification of the context and the list of potential hazards, also known as *context definition,* and the subsequent stages where these are evaluated. Each part has an icon associated with it that ties the relevant parts of the figure to the text.

Determining the context is the first step in problem formulation, and includes the receiving environment and the GMO's intended use. It also includes information on the gene construct used to make the GMO, and its biological characteristics. It is important to consider the GMO's expected behavior in the environment, as well as the way the GMO reproduces and propagates, and the comparators' biology and its uses.

The context also includes identifying the protection goals, i.e., the plants, animals, or other ecosystem components that are being protected from possible harmful effects of the GMO. Protection goals can be specific, e.g., a given endangered species, or general, such as 'the environment' or 'the forest'.

Protection goals are defined by the country's legal framework. Because protection goals depend on the legal framework, the GMO's biology, and the receiving environment, it is not possible to formulate a list of specific protection goals applicable to all cases. It is, however, possible to identify the general topics that frame the questions for each case. Accordingly, several case studies (one of which is included in Part 2) to show how the assessment principles have been applied in different situations. If properly conducted, problem formulation helps ensure that the ERA is relevant for decision-making (Wolt et al., 2010*).*

Finally, it should be recognized that usually it is not every impact of a GMO that is being assessed, but only those effects that differ from equivalent non-GM organisms (the "comparator") in a given environment, and which serve as indicators of potential and likely harm compared to the conventional counterpart. It is important to note that the cultivation of any conventional crop and agriculture in general can have negative environmental impacts. Therefore, for most jurisdictions, the purpose of an ERA for a GM crop is not to determine the impact of agriculture in the receiving environment; the objective is to determine whether the genetic modification has resulted in changes in the crop that can lead to worst environmental impacts than the conventional crop. Ultimately, each ERA is framed in the context of its specific national legislation.

a. Identification of the protection goals relevant to the assessment. Protection goals are set by policy and will depend on the legal framework of each country. These policy protection goals tend to be very broad (e.g., protection of biodiversity, protection of human and animal health, etc.) in order to protect as many aspects as possible, and are usually formulated in legal terms using concepts such as 'sustainability,' 'integrity,' 'acceptability,' etc…. Such broad statements can be widely interpreted and are often impossible to prove true or false, as they are too vague to be scientifically assessed (Garcia-Alonso and Raybould, 2013).

Environmental risk assessments use a scientific approach to identify and assess protection goals. Specific biologically possible hypotheses are formulated and tested to determine if these can be falsified by appropriate testing methods. Therefore, policy protection goals need to be translated into specific protection goals that can be used in the risk assessment. (Garcia-Alonso and Raybould, 2013).

The relevant specific protection goals for an environmental risk assessment may differ from country to country and product to product, so it is not always possible to formulate a list of specific protection goals applicable to all cases. However, it is possible to identify the general topics or areas of assessment that are commonly addressed by environmental risk assessments, such as: protecting beneficial species; preserving or improving water and soil resources; protecting iconic and endangered species, and protecting the country's genetic resources (e.g., landrace varieties).

Biodiversity and protection goals

A fairly universal protection goal is the protection of biodiversity. However, biodiversity is a very broad and complex concept. To conduct an ERA that will address this protection goal, it is first necessary to identify those aspects of biodiversity that could be harmed by the use of a given GMO, but are not harmed by the conventional counterpart. It is also important that this harm can be measured. This allows the formulation of specific protection goals from which *assessment endpoints* can be derived.

An assessment endpoint is defined as whatever will be measured to determine if the protection goal is being protected as intended (Sanvido et al., 2012). For example, pollinators are a valued ecological function in agricultural production. Therefore, pollinators are a protection goal whenever insecticidal plants are deployed. The effects of the newly expressed insecticidal proteins in the GM crop can be tested on a representative pollinator, usually honeybees, to assess for potential adverse effects on pollinator populations. Therefore, the susceptibility of a given honeybee population to the insecticidal GM crop represents an assessment endpoint, which is measurable, and represents a larger protection goal, namely pollinators.

At the same time, it makes no sense to use bee numbers as an assessment endpoint for a GM crop that has altered oil quality, or any other trait that cannot possibly harm bees. All too often 'universal' endpoints—e.g., bees, earthworms, and some fungi are used that poorly represent the protection goals that could be harmed by the GMO. The use of such 'universal' endpoints increases costs without contributing to the value of the ERA.

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b. Knowledge of the comparator's biology and its uses, with emphasis on describing those aspects of the comparator that may help predict the GMO's behavior. This is why most dossiers start with a detailed explanation about what is known about the conventional crop. In addition, there are many published sources that provide information on the biology of conventional crops, their cultivation, and their uses (e.g. peer-reviewed papers and international consensus documents such as those published by OECD). For the purpose of the ERA, this information provides the

baseline to use in the comparative assessment to determine if the genetic modification has resulted in change to the crop that could lead to harm and to identify potential hazards.

The reproductive biology of the conventional crop is a key component that must be considered when determining the risks. For example, if a conventional crop can cross with sexually compatible wild relatives, the GM version could also cross, thus, the likelihood and the consequences of the crossing should be evaluated during the risk assessment. While some crops die after setting seed (annuals), others can survive for many years (perennial), which means they can produce seeds and pollen for many years.

Other aspects of plant reproductive biology to consider during an ERA

Certain crops primarily self-pollinate (autogamous), so their pollen is not very mobile. Other crops readily outcross (allogamous), and their pollen can be transported by wind, insects, or even birds or bats, increasing the distance that the pollen can disperse.

Another important dispersal mechanism is through seeds, which can be carried by wind, water, some animals, farm equipment, and humans through some cultural practices. Some seeds can survive for many years prior to germination.

Just because a plant has the ability to flower and set seed does not mean it will. Some crop varieties (e.g., sugarcane) will only flower under the right conditions of daylight hours.

Certain crops are propagated vegetatively for cultivation by grafting, cuttings, buds, and rhizomes. However, some also have the capacity to produce pollen or seeds, and tissues with reproductive capacity can persist in the soil for extended periods or be exchanged by small holder farmers in tropical settings.

Finally, there are crops such as the cultivated bananas, which are sterile under all conditions and propagate only vegetatively. In such cases, there is no pollen or gene flow, but reproductive tissues can remain in the ground for extended periods

c. The receiving environment and intended use. The receiving environment where the GM crop will be used is considered during problem formulation. A conventional crop may have different agronomic behavior under different environmental conditions. A crop may not be able to survive the seasonal temperatures and biotic conditions in one country, while it could in another country. Also, the receiving environment may have characteristics that influence the pathways of exposure. For example, a crop may be grown in a

country where no sexually compatible wild relatives are present. In this case the risk that the trait introduced in the GM crop is transferred to sexually compatible wild relatives resulting in environmental harm (e.g., increased fitness of the wild relative resulting in displacement of valued protected species) is negligible (no exposure). The same crop grown in another country where sexually compatible wild relatives are present may pose a risk that may need to be evaluated.

The biology, ecology and agronomic behavior of the conventional crop in the context of the specific receiving environment are thus considered during problem formulation. It is also important to consider the intended use of the GMO, as it determines the scope of the ERA.

For example, if the ERA is conducted to estimate potential adverse effects of the release of a GMO for the purpose of conducting a research field trial, environmental exposure can be minimized using standard practices. The trial could be placed in areas where cross-pollination would be minimal, physical barriers such border rows could be used or the crop could be planted at a time where receptive plants surrounding the trial would not be ready to receive any pollen shed. This exposure management would then greatly reduce any risks identified. The measures implemented will depend on the crop/trait/receiving environment combination.

In addition, as described earlier, this part of the ERA considers national protection goals, which in most countries include the protection of biodiversity, and often the protection of agricultural production itself. Therefore, whenever a GMO is expressing a toxin not found in the conventional version, it is useful to identify non-target organisms, that is, valued organisms present in the receiving environment that could be harmed by the use of the GMO, resulting in loss of biodiversity or harm to important ecological functions (e.g., pollination, biological control, or decomposition).

d. Genetic construct and transgene expression. Regulatory dossiers contain a section that describes the transgene and the way it was put together. Accordingly, certain information on the construct used to produce the GMO can be useful for identifying potential hazards and for anticipating exposure. For example, there are some genes that have been used for the production of different types of GM crops, such as Cry1Ac, which has been used in cotton, maize, soybean,

and eggplant/brinjal, CERA, 2010). Sequence analysis of the inserted gene could confirm if the sequence of the gene is the same in all the crops and likely to express the exact Cry1Ac protein. If so, any information generated with the protein in isolation would be relevant for the ERA of all these crops. If toxicity or ecotoxicology studies have been conducted, there may be available information that suggests what organisms may be at risk, so the ERA can focus on those. Also, the information may be used to dismiss some potential risks, such as when a hazard to a particular group of organisms has not been found (e.g., honeybees, so it is a pre-ordained conclusion that the risk will be negligible).

Information on other gene components is also considered, such as promoters. This may provide some clues on potential patterns of expression from which exposure patterns can be anticipated. For example, the exposure will be different if the transgene is expressed continuously or temporarily, or if it is expressed throughout the plant or just in certain tissues, such as roots.

The source organism of the gene is always taken into consideration, although this information is of primarily important for food and feed safety assessment, on the premise that the undesirable characteristics of some organisms (e.g., the ability of peanut/groundnuts to cause allergies in some people) could have been transferred into the recipient GMO; it is therefore important to verify that the transferred gene is not responsible for the source organism's undesirable characteristics. E.g., if a gene from peanut/groundnut was to be used, it would be important to ensure it was not responsible for causing allergies.

Finally, other information provided, such as the construct map and elements that constitute the vector used for transformation, and the restriction sites of enzymes, do not provide useful information for the ERA. This information may be useful for designing detection methods, but for the ERA the emphasis is mainly on the phenotypic characteristics of the GMO.

Concept of familiarity in the National Research Council of the US Academy of Sciences

Since the beginning of agriculture, almost all crops have been genetically modified through conventional methods, using selection, mutagenesis, and crossbreeding. It is important to note that all potential environmental impacts evaluated in the ERA must be related to the *trait* conferred by the modification, and not to the *method* (conventional or rDNA) used to make the change. For this reason, it is appropriate to use the historical behavior of the comparator to help predict the behavior of a GMO under certain circumstances, according to the concept of familiarity developed the U.S. National Academy of Sciences (NAS, 1989), which concluded that "crops modified by genetic engineering should not present risks different from those presented by crops modified through conventional plant improvement for similar traits and cultivated under similar conditions."

This concept also applies to changes caused by transcription factors or by gene-silencing, which in turn can regulate the expression of many other genes. Conventional breeding frequently uses traits due to mutations in regulatory genes that are normally expressed under certain conditions (Parrott et al., 2010). In these examples [e.g., fruit size in tomato, seed retention in wheat, height of wheat plants, the single-stalked modern maize plant], no indications have been found that modifying regulatory genes poses risks different from those present through conventional breeding.

Concept of gene flow

Both GMO and conventional crops can cross with other varieties of the same crop, and sometimes with other sexually compatible species. This natural process is called gene flow whenever the gene remains in the new population. Otherwise it is simply pollen flow. Unfortunately, the concepts of gene flow and pollen flow are often confused and used interchangeably.

Crossing between varieties of the same crop is a fact that farmers and seed producers have been managing for centuries. For conventional seed production, measures are taken to minimize crossing with other varieties and thus maximize seed purity. Farmers that grow distinct varieties also know how to minimize crossing from nearby varieties that can compromise the purity or quality of their production.

Crossing between a conventional crop and its wild and feral relatives can occur whenever they grow in close proximity. Crossing with sexually compatible wild species can also occur naturally, but tends to be less common. There is a perception that interspecific crosses are easy, as crop breeders resort to these crosses as a source of genetic diversity to create improved crop varieties. The fact that breeders must usually resort to extraordinary measures to facilitate these crosses (e.g. laboratory procedures like embryo rescue or protoplast fusion) is generally not recognized. Regardless, when interspecific crosses do happen and gene flow ensues (introgression, to use breeding terminology), the acquisition of new genes can contribute to natural selection and evolution.

Whenever pollen or gene flow is associated with GM crops, they are frequently perceived incorrectly as an automatic environmental risk. The crossing of a GM crop with a conventional variety is sometimes referred to as “contamination”. This type of crossing is very unlikely to lead to environmental harm; it is a commercial issue that can be managed in the same way that the coexistence of different conventional varieties of the same crop or seed production has been managed for years.

The movement of a newly inserted gene from a GM crop to

a sexually compatible wild relative is also perceived as an environmental risk and is often addressed in ERAs. The main concern is that the new gene could become established in the wild relative population, conferring a new trait that could provide it with a selective advantage that could lead to environmental harm. The plant could have increased weediness, leading to greater invasiveness potential and displacement of native valued species.

It is important to note that the occurrence of gene flow “per se” from a GM crops is not an environmental harm. It is the consequences of the establishment of the gene in other populations that is the focus of the assessment.

There are a number of events that must occur before a gene from a GM crop is established in another population:

1. Crossing with sexually compatible species or other varieties of the same species; for this to happen, both must be geographically closely located and present similar flowering time. In addition, the progeny must be viable.
2. The gene must be present in successive generations (introgression).
3. In general, for the gene to remain in the population, it needs to give a competitive advantage to the progeny (e.g. pest resistance); the exception is swamping—which is what happens when the sheer number of crosses between two populations ensures the gene remains in the new generation.

The fact that gene flow (and persistence of the gene in the population) takes place does not automatically mean that there is a hazard. Consequently, it is necessary to evaluate the possible effects that the presence of this gene may have on the species to which the gene was introduced by crossing, and its interaction with other organisms in the environment. These concepts are further developed in Figure 6.

A specific case that illustrates both the concept of crop familiarity and gene flow is that of maize and its progenitor, teosinte. Teosinte has been growing close to maize for millennia, and maize landraces have been planted side by side for several centuries. Although it is well documented that maize and teosinte cross, as do the various landraces

of maize, and although there is evidence that such pollen flow has resulted in gene flow, this gene flow has not damaged teosinte or the several landraces of maize, all of which retain their identity.

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e. History of safe use. In some cases there is information available from the cultivation of a GM crop in other geographical regions, in which case the summary and conclusions available from other assessments can provide useful information that can be considered during problem formulation. When the GM crop has been used for many years in other countries, there is a history of safe use that contributes to the weight of evidence for the ERA. While there are no two completely identical environments, there are environmental conditions that are comparable, which allow inferences about expected results. Also, there are many evaluations conducted during the ERA that are done in controlled laboratory conditions and thus can be used in any geography (e.g., ecotoxicology laboratory studies).

It is important to remember that the ERA for GM crops uses a comparative approach, thus, most of the evaluations conducted under field conditions focus on the like for like comparison between the GM crop and a conventional comparator, trying to establish whether the genetic modification has led to differences that can lead to harm. This comparison is often performed in different environments to determine whether environmental conditions influence this comparison. Once the comparison has been conducted in a number of different environments, if no differences other than the intended trait have been observed, it is unlikely that evaluations under other environmental conditions will reveal new differences that could lead to harm (Garcia-Alonso et al., 2014). There is now a wealth of evidence that supports that genetic modification using recombinant DNA is no more likely than conventional breeding to result in unintended changes that can lead to novel harm (Weber et al., 2012).

Risk assessments and data transportability

Article 13 of the Cartagena Protocol foresees that Parties may adopt simplified procedures for the authorization of GMO imports. According to Article 13.1 (b), the Parties may even exempt certain GMOs from the Advanced Informed Agreement (AIA) procedure.

In other words, the Parties can use risk assessments carried out in other countries when they prepare their own ERA. If the authority responsible for risk assessment of another country Party to the Protocol dealt with similar environmental risks, another Party can accept and rely on the risk assessment that has already been made by the first Party. In addition, Parties can also use the risk assessment from another country to clarify and narrow the range of hazards identified to avoid duplication in the risk assessment.

It is generally accepted that through the adoption of simplified procedures under Article 13 of the Protocol, the Parties are also able to comply with the Sanitary and Phytosanitary (SPS) Measures Agreement of the World Trade Organization. Under Article 4 of the SPS Agreement entitled “Equivalence”, the Parties undertake mutual recognition of equivalent standards in other countries. Through the use of Article 13 of the Protocol to recognize a risk assessment conducted by another country, the Parties comply with their international obligations within the framework of the Protocol and within the framework of the SPS Agreement.

As a complementary form of information acquisition in which to base GMO risk assessment, Parties can use the information shared with the Center for the Exchange of Information on Biosafety (better known as the Biosafety Clearing House, BCH), implemented under Article 20 of the Protocol, where data is available on risk assessment already carried out and on GMOs approved for commercial release in other countries.

3.3 Stage two: Problem formulation: hazard identification and characterization

Once the context is established, the next step is to compile a list or inventory of all potential hazards that could occur if the GMO was released into the receiving environment. At this point, the evaluator should not try to assess the risk associated with each hazard, or formulate a hypothesis that could explain them, but simply list hazards that may be associated, , with the introduction of the GM in the receiving environment. The hazards can be identified from the experience of specialists, as well as from concerns of the general public. The context will subsequently provide the information that is used to determine which of the listed hazards merit further assessment. A comparative assessment is done following a "weight of evidence" approach, in which information from different sources is considered to determine those differences between the GM crop and the conventional crop that can lead to harm and must be assessed (i.e., hazard identification). The result may be another set of potential hazards that is added to the risk assessment.

In cases where the GMO has been previously assessed by other risk assessment agencies and has a history of safe use, the evaluator can start with a list of hazards that has been narrowed down based on previous experience with a similar GMO, or based on scientific data from other sources.

Common categories of environmental risks

Since the purpose of the ERA is to facilitate decision-making, the protection goals set in the country are considered first. As discussed before, these protection goals can differ from country to country, but in general there are some areas of assessment that are often considered in the ERA. For example, potential effects on beneficial organisms in agricultural fields or effects on species of conservation value in natural habitats, weediness potential, etc. While specific questions are different for each GMO, there are general categories to be considered. As an example, there are hazard categories (potential adverse effects) that may be associated

with a GM crop.

General concerns associated with a GMO

Increased persistence and invasiveness, or "weediness potential." The concern is that the genetic modification will make the crop more "weedy" and difficult to control in an agricultural field, or more invasive in natural habitats outside the farm, thus displacing valued plant populations and causing a decline in biodiversity. Alternatively, the new trait could be transferred to sexually compatible wild relatives, turning them into more persistent weeds in agricultural fields or making them more invasive in natural habitats, also leading to a decline in biodiversity. Specific traits that lead to increased fitness or adaptation of the GMO to different environments include:

- Higher fertility rates
- Invasive behavior
- Survival under greater stress conditions

Typical risks associated with the production of new toxins in a GMO

The concern is that a GM crop designed to produce a new toxin will have an adverse impact on beneficial organisms associated with the crop and that contribute to valued ecological functions such as pollination, biological control or decomposition. In addition, there is a concern that the GM crop could have adverse effects on species of conservation value that may live in agriculture fields or in surrounding natural habitats. Non-target organisms include:

- Arthropods
- Other invertebrates, especially in the soil
- Vertebrates
- Soil microorganisms

Other possible impacts include adverse effects on important ecological functions that contribute to sustainable agriculture. The concern is that the cultivation of the GM crop will compromise the productivity of the field in subsequent seasons due to adverse effects on soil, such as by:

- Accumulation of non-inactivated toxins in the soil that can harm important soil functions.
- Changes in the biodegradation rate of the GMO by-products

Risks associated with an herbicide-tolerant GMO

Adverse effects on the environment due to changes in associated management practices, such as:

- Promotion of undesirable agronomic practices (e.g., lack of

rotation)

Whereas weediness or toxicity are universally accepted as potential harms, concerns over changes in cultural practices, potential for resistance development and other systems issues are country-specific.

Risks to the effectiveness of GM technology

These do not cause damage to the environment, and are a commercial issue, not an issue that is treated within an ERA. These include:

- For insect resistance traits
  - Selection of resistant pests
- For herbicide tolerant traits
  - Selection of resistant weeds

The evolution of resistance to control measures has been a problem in conventional agriculture for the past century. Although it is assumed that pests and weeds can develop resistance to conventional pesticides, this is not part of the pre-commercial risk assessment. It is dealt with by implementing good agronomic practices and stewardship programs that may help in delaying the emergence of such resistance. More information on the concept of stewardship is in Chapter 4.

3.4 Stage three: Exposure characterization

Risk is a function of hazard and exposure, therefore it is important to determine not only the potential hazard that the GM crop may pose, but also the level of exposure of valued organisms or processes that could be at risk. For this, information gathered during the context step is useful. The level of exposure will vary greatly depending on the proposed use of the GM crop. If the ERA is for a field trial release, exposure will be limited spatially and temporally and can be managed through standard field trial techniques. If the ERA is for a cultivation release then exposure has to be characterized.

For some organisms, it may be impossible to predict exact levels of exposure to a GMO following a commercial release. In some cases the levels of expression of the gene products in tissues of the GMOs can provide an estimate of exposure to a particular group of organisms or processes. A common approach used in risk assessment is to consider “scenarios of exposure”, where the ERA estimates what would be the maximum level of exposure or “worst

case scenario". This allows making predictions on potential risk. If under a worst case scenario of exposure the risk that a hazard will cause environmental harm is low, under more realistic exposure the risk will be even lower.

The information gathered on, and the experience with the conventional crop and the receiving environment, are useful to establish the organisms that may be exposed to the GMO and could therefore be at risk. Information gathered on the gene product can also be useful, especially for those proteins that have been used previously studied and for which information regarding toxic effects on various organisms is known. In addition, expression levels of the newly expressed proteins in different parts of the GM plant can be used to have a more detailed characterization of exposure. For example if one of the assessment endpoints is to determine potential risk to pollinators, it is important to know whether the newly expressed protein i.e., potential hazard agent is expressed in pollen and at what level.

Exposure can be either estimated using quantitative approaches (e.g a herbivore feeding on a plant tissues expressing x amount of protein will be exposed to x levels) or qualitative approaches (e.g. exposure is unlikely or exposure could occur in some circumstances). Whatever the approach this provides an estimation that can be used to characterizing the risk when consider the levels of hazard.

3.5 Stage four: Risk characterization

The next step in risk assessment is risk characterization, i.e., determining which hazards actually pose a risk, i.e., truly have the ability to cause harm under the proposed use of the GM crop. In this step, each of the hazards listed previously and exposure are considered. The focus is to evaluate the probability that harm may occur and the magnitude and significance of the consequences to the environment if it occurs.

This is done using the data compiled during problem formulation (i.e., biology, ecology and agronomic characteristics of the recipient crop, the genes used for the modification, the intended use, the receiving environment, expression levels and the history of safe use).

Not all potential risks considered will be relevant for the risk assessment of a particular GMO. Some will not have a biological basis given the nature of the GMO. For others, it may not be possible to establish a causal relationship that relates them with the GMO. In some cases, it can be established that exposure to certain organisms is unlikely to occur, or that the trait in the GMO is not likely to pose a hazard to certain organisms. For example, if there are no sexually compatible wild relatives of the crop in the country where the ERA is conducted, it can be concluded at this step that there will be no exposure; therefore the risk to wild relatives will be negligible.

Figure 11: Conventional (top) and transgenic (Bt, bottom) maize growing in San Antonio, Honduras.

Photo by MM Roca.

For a GMO to harm the protection goal, there has to be a series of events that connect the two together, referred to as the *pathway to harm.* The pathway to harm must establish the cause-and-effect relationship between hazard and harm. The identification of events that must take place for harm to occur facilitates the assessment. If all of the steps in the pathway can occur, then the magnitude of the consequences must be assessed. This assessment is always done in a comparative manner, taking into account the behavior of the conventional crop.

Using this approach, if it can be established that one of the steps is not plausible and cannot occur, then the pathway is broken and it can be concluded that the risk will be negligible. There is no need to generate information for the rest of the steps, as doing so will not provide more information to characterize risk. This is valuable tool for transparency and risk communication, especially in situations where risks were considered during the assessment process but were ruled out due to lack of a plausible path for its occurrence. Figures 7 and 8 show examples of how path to harm is established to determine if there is a causal relationship (a path to harm) between a hazard and its associated damage for two different cases. Each

individual step in the pathway to harm should be amenable to hypothesis-based testing.

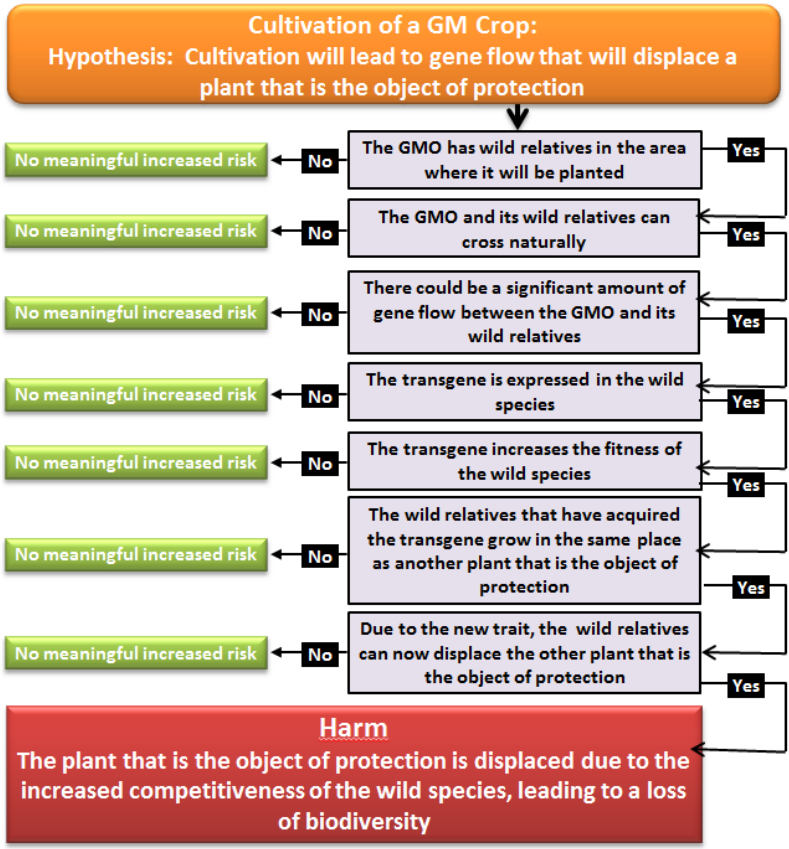

Figure 12: The pathway to harm from gene flow and the competitive advantage that could be conferred by genetic modification. This diagram shows all the events that need to occur for gene flow to cause harm, highlighting the parameters used to assess possible effects, in this case, on a population of interest, based on the hypothesis that "Pollen flow from the GM crop will result in the establishment of the new gene in sexually compatible wild relatives displacing populations of a given plant" which is the final assessment endpoint and protection goal.

It is very important to note that there could be many pathways to harm for any given trait, and that it is impossible to assess every one of them (e.g., pathways to harm for every single non-target

organism at risk). It is important to focus on one pathway that is informative for the risk.

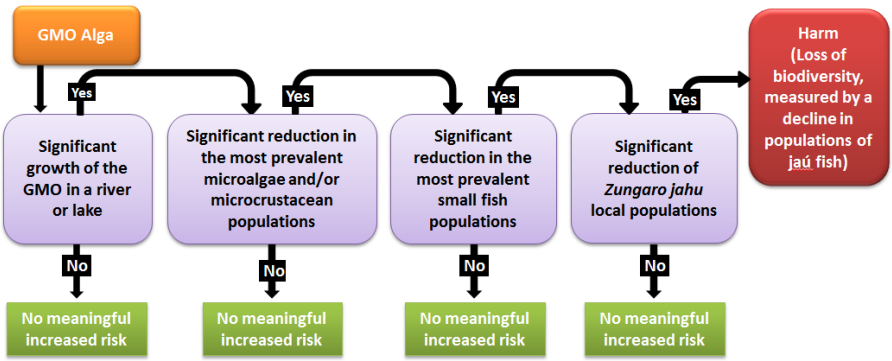

Figure 13: The pathway to harm from a GM alga. This diagram shows all events that need to occur for the GM algal introduction to cause harm, highlighting the parameters to be evaluated to assess possible effects, in this case, upon the jaú fish population, which is the object of protection that is used as an assessment endpoint.

Once the pathway to harm is established, the likelihood of harm resulting from exposure to the GMO is estimated using the assessment endpoints. Therefore, in this step, the two actions (estimating exposure, and if any, the determination of the consequences of this exposure, i.e., the new harm associated with a GMO but not produced by its non-GM counterpart) are considered together to estimate risk.

Figures 7 and 8 also help clarify the concept of assessment endpoints. A single assessment endpoint can address the protection of different ecological entities. In other words, an indicator species can be used to represent other similar species, e.g., one bird versus each single species of bird, (unless a specific species is the main object of protection).

When selecting assessment endpoints, it is important to recognize that the complexity of the context does not necessarily require that all the variables get measured. For example, the negative environmental impact due to the cultivation of a plant resistant to the

attack of certain insects by the production of an insecticidal protein (e.g., Bt), could be assessed using several different parameters (e.g., the multi-trophic impact on birds that eat insects that parasitize a pest that fed on a GM crop), in addition to those truly relevant variables (e.g., the impact on a susceptible non-target organism). However, the additional multitrophic data would not be very informative.

Selection of one or more non-target species that could adequately represent the risk can be difficult, but at present, there is extensive experience in this area of risk assessment (See box below). Conversely, relevant information can be obtained from evaluations carried out in other countries, provided that test conditions are consistent with those in the countries that want to adopt the technology.

Toxicity and non-target organisms

A common misconception about toxins is that they are assumed to be toxic to every organism. They typically are not. First, many toxins are only toxic to certain species, genera, etc. For example, theobromine, a compound found in chocolate, is essentially nontoxic to humans, but a few bars of chocolate contain enough theobromine to kill a cat or a dog. Therefore, the choice of organisms whose eventual susceptibility will be assessed (i.e., assessment endpoints) depends on the mode of toxicity introduced by the GMO, the range of susceptible species, and the toxicity and amount present of the toxin present. Toxicity is dose-dependent--there will only be harm if the dose is high enough.

> For example, a particular Bt crop produces toxins that harm only certain insect families within the Lepidoptera or Coleoptera. In contrast, if lectin (a plant derived toxin) were to be used, it could be toxic to a wider range of arthropods and vertebrates.
>
> Finally, an organism's susceptibility to a toxin produced by a GMO does not result in harm if the susceptible organism is not exposed to the toxin. For example, a caterpillar may be widely exposed to the toxin in the leaves of a GM crop, but a nectar consuming insect or a susceptible aquatic insect may not be exposed. Alternatively, the caterpillar may not be present at times when the crop is in the field, so there would be no exposure.

During this step the evaluator may find that he or she requires specific information that was not initially provided. In all cases, reports published in prestigious, peer-reviewed books and journals are useful sources of additional data. If the information is still not readily available, the generation of data in laboratory or field studies (see box on Lack of data and the need for confined trials) will be required. The need to conduct these studies is determined during problem formulation where assessment endpoints have been established. A

publication by Garcia- Alonso and Raybould (2013) provide some examples.

If the need to conduct additional laboratory or field studies is identified, the tests are carefully design to provide answers to specific test hypothesis. For example, for non-target organisms, the species to test are carefully selected to represent groups of organisms for which a potential risk has been identified (i.e., organisms that can be exposed or could be affected by the trait) and for which robust testing methodology exists. *It is important to note that it is not necessary to test all organisms present in the receiving environment.* In the same way as rodent tests are conducted to predict effects in humans, certain species of arthropods are used to predict effects on taxonomically related species or species from the same functional group, such as the use of honeybees to represent pollinators (Romeis et al., 2012).

Questions that are purely speculative or that only answer to the curiosity or concern of a particular person or group are not appropriate or relevant for risk assessment.

Lack of data and the need for confined trials

It may be the case for new GMOs that there is not enough information available anywhere (see box on Data Transportability) to adequately evaluate its potential harm to the intended receiving environment.

Generally, the safety evaluation of GMOs starts with field trials conducted under confined conditions, where the conventional crop is used as a comparator. These trials help to determine if the GMO exhibits unexpected behavior (unintended differences). In turn, data obtained from these observations is used to inform the ERA.

Out of necessity, confined releases are made *prior* to the completion of the ERA, to collect data for regulatory submissions or when it is considered that further data is necessary.

Confined releases are approved after appropriate measures are put in place for confinement of the GMO. Confinement measures might include, for example, minimum separation distances between the GMO field and conventional fields,

different planting dates -for asynchronous flowering, or the elimination of reproductive structures, (flowers, rhizomes, etc.), crop destruction methods and monitoring the field during subsequent plantings to remove plants from misplaced seeds that germinate ("volunteers"). If fields are mechanized, exclusive machinery is used in GM fields, or the machinery is cleaned thoroughly before using it in a conventional non-GM field.

The steps to be followed depend on country regulations. In some countries, if problems do not arise during confined release trials, commercial use may be approved. In other countries, GMOs go to a semi-commercial stage before reaching the commercial stage.

The need to conduct a local confined field trial should be determined during problem formulation. If considered necessary, the confined field trial must be carefully designed to provide the data needed, as data from confined field trials can often be difficult to interpret.

3.6 Stage five: Risk estimation

The fourth stage of the risk assessment is to produce an estimate of risk based on the likelihood that a risk will occur, and if any, the harm that would result as determined by the assessment endpoint associated with the protection goals.

This estimate is always relative, in that it is always compared to the way in which the conventional organism affects the same endpoints assessed under the same conditions.

For some areas of assessment, a quantitative approach can be applied. For example where exposure and hazard can be measured, toxicity exposure ratios can be calculated and compared to thresholds established by regulations to determine if the risk is acceptable. This approach is widely used for pesticides and chemicals, but in the case of GMOs there is no consensus on what the thresholds would be or what levels of risk would be acceptable.

For most areas of assessment, a quantitative measure of exposure is not feasible. Therefore, the use of qualitative measures is suggested, as shown in Table 1 (OTGR, 2009).

Table 1. **Categories used to estimate the likelihood of an event happening**

Likelihood	Assessment definitions
Very high	Is expected to occur in most circumstances
High	Could occur in many circumstances
Low	Could occur in some circumstances
Very low	May occur only in very rare circumstances

Likewise, there will be cases for which a quantitative measure of harm to the environment is not possible, but qualitative measures can be used. An example is shown in Table 2. For both exposure and harm, whenever qualitative measures are used, it is important to ensure consistency in the description of the qualitative categories to facilitate communication among stakeholders (e.g. risk assessors and risk managers). In all cases, the determination is based on the assessment endpoints for the protection goal, which in turn is a proxy for the environment, including its biological communities.

Table 2. Categories used to estimate the amount of harm if the event were to happen

Consequences	Impact on the protection goal
Marginal	Negligible or no harm
Minor	Harm that is reversible and limited in time and space or numbers affected
Great	Widespread harm and disruption of communities of the protection goal, but reversible or of limited severity
Major	Extensive harm and disruption that persists over time or is not readily reversible; the object of protection could become extinct

Combining Tables 1 and 2 results in Table 3, which makes it possible to classify the risk using qualitative categories, taking into account the likelihood of occurrence and the consequence assessment:

		RISK			
PROBABILITY	Very high	Low	Moderate	High	High
	High	Low	Low	Moderate	High
	Low	Negligible	Low	Moderate	Moderate
	Very low	Negligible	Negligible	Low	Moderate
		Marginal	Minor	Great	Major
		Harm			

***Table 3*:** Matrix for qualitative risk estimation due to the introduction of a GMO into a receiving environment. Estimates of harm (resulting from t) and probability (or exposure frequency and magnitude) should be made based on the information located in the risk characterization stage (adapted from OTGR, 2009).

To date, the risks assessed for commercially authorized GMOs have been considered to be negligible in the sense of not having a negative environmental impact that differs from the impacts of not-GM counterpart on the same organisms. Therefore, as shown in Table 3, either the consequences have been assessed as marginal or minor, or the probability of harm occurrence has been considered to be low or very low.

Multiple probabilities in a pathway to harm

A property of probabilities is that any probability less than1 results in an even smaller probability whenever multiplied together. The concept can be illustrated by assigning probabilities to Figure 8, as illustrated in Figure 9. Thus, if one event has a 10% likelihood of happening, and another one has a 90% chance of happening, the chances of both happening are just 9% (mathematically, 0.1 x 0.9 = 0.09). If each step in the route to harm has less than a 100% chance of happening, harm resulting from the pathway is a very unlikely. Note that it is not necessary to assign quantitative values to each step. Low probabilities always get lower when multiplied together.

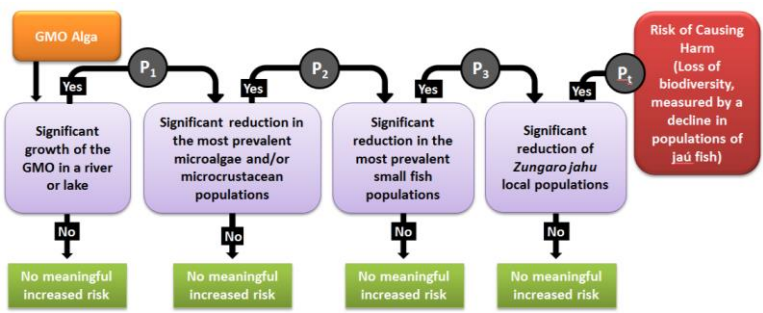

Figure 14: The pathway to harm from a GM alga, modified to assign a qualitative probability to each part of the pathway to harm.

3.7 Stage six: Recommendation

Once the potential risks associated with the release of a GMO in the environment have been estimated, the assessor can make a recommendation as to whether the risk is acceptable or not. Accordingly, typical recommendations are to release without conditions, release with conditions (field trials), or that more data are needed.

Risk assessment is a technical job – it assesses the risk to the environment, and estimate the probability of risk. After that, the risk assessor usually only submits recommendations and does not make decisions.

Most often, the developer of the GMO conducts the risk assessment, and the in-country regulators simply audit the risk assessment dossier to ensure that the assessment was conducted properly. In-country regulators do not need to repeat the assessment nor do it for the developer.

The decision-making process is carried out differently according to the legislation of each country. Regardless of country-specific differences, the appointed decision makers take the recommendation from the scientific risk assessment and, decide on the acceptability of risks assessed, define risk management measures if needed, and at times may consider other aspects beyond risk assessment, such as socio-economic considerations to make decisions. This is what is referred to as “Risk management” which is the next step after the risk assessment in the Risk analysis process.

In fact, the Cartagena Protocol includes the option to incorporate socio-economic considerations in risk-related decisions. However, it is important to remember that socio-economic considerations are not included in the risk assessment, they are considered later in the risk analysis process to help make the final decision on whether to approve or not a product. This approach has been adopted by some countries; for example, Argentina, once the risk assessment has been evaluated scientifically, considers among the criteria used to approve a genetically modified organism, the possibility that their exports may be adversely affected by cultivating that GMO.

In summary, risk assessment is a tool that applies scientific methodology to determine the likelihood that harm could occur and the magnitude of the consequences if harm occurred. Although it appears as complex and multifaceted, this process is facilitated by the use of problem formulation that determines the relevant protection goals and assessment endpoints and provides a logical and transparent way to conduct ERAs.

3.8 Synopsis

The ERA five stages are graphically summarized in Figure 10:

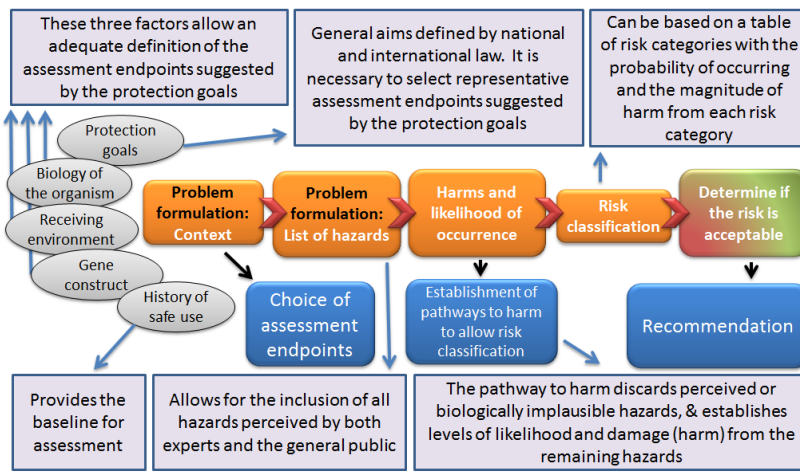

Figure 15: Summary of the risk assessment process as covered in this chapter and the purpose for each of the parts.

Chapter 4: Risk Management & Communication

Risk management consists of options to reduce or avoid novel environmental risks identified in the risk assessment. Risk management plans are carefully designed for practical and effective adoption of mitigation measures.

Measures for risk management are determined on a case-by-case basis whenever they are considered necessary. In most cases, there will be no need to take risk-mitigating management measures; in other cases, specific measures may be justified. Risk management also leads to communicating the risk to the section of the public that could be affected by GM, and it also feeds back damage-related information to the manager.

In some countries, even when no specific risks were identified during the risk assessments, generic risk management measures have ben implemented as a tool to appease public perception. It is worth highlighting that so far, not a single GMO evaluated and approved by existing national regulatory bodies has led to adverse environmental effects caused by the genetic modification.

Any risk management measures undertaken should always be specific to a given GMO and proportional to the identified risk. For example, Brazil imposed an exclusion area for GM cotton cultivation due to the existence of a native, sexually compatible cotton species in that area.

Other risk management measures are taken on the basis of socio-economic considerations rather than environmental safety. For example, in Brazil, to ensure coexistence among different types of maize, measures such as minimum distances between crops and delayed planting programs have been established to reduce pollen flow. When growing GMOs for the production of therapeutic proteins, risk management measures are much stricter; crops must be established in remote or confined areas and require that the harvest be handled in closed warehouses and containers to ensure their contained use.

Post-marketing monitoring

When a risk can not be ruled out in the ERA, authorities may still approve the commercial release of the product, but request a monitoring programme specifically focused on that risk. The monitoring is then aimed at testing a a defined risk hypothesis, for example, the development of resistance of an insect pest to aBt crop.

In contrast, general monitoring without focusing on a specific risk lacks scientific basis, and no hypothesis can be formulated to help with its assessment. Therefore, if harm occurs, it is impossible to establish a cause-and-effect link. Article 16 of the Cartagena Protocol allows Parties to adopt risk management measures. Although post-marketing monitoring may be an appropriate risk management measure for some cases, Article 16 does not require Parties to adopt it; Parties may exercise their own judgment about when and if to adopt monitoring within the risk management context.

Coexistence

Coexistence is not an environmental risk issue; rather, it is a socio-economic, commercial or even stewardship issue. Since the beginning of the seed industry more than one century ago, the need to maintain pure varieties has been recognized. It was difficult to obtain pure seed for varieties that cross easily due to open pollination. To keep seed as pure as possible, separation distances were established during seed production. However, it is not practical or realistic to completely prevent cross-pollination. Therefore, thresholds also were established for the presence of seeds from one variety in another. For example, in some cases, varietal purity is determined in the field, and in most cases, no more than one or two atypical plants per 100 plants are permitted. These levels are low enough not to affect the characteristics of any given variety. The use of thresholds allows the coexistence of seed production of several varieties, and seed production with neighboring agricultural production. The use of thresholds allows the coexistence of different varieties of a crop, and also allows the coexistence of different types of farming, like conventional or organic with GMO farming. In contrast, zero tolerance (with no thresholds) can only be achieved by forbidding the use of a variety or of a type of farming, with strict controls to ensure that bans are observed. In other words, there is no coexistence.

Stewardship

Adopting a GM crop for marketing may involve restrictions or conditions. Some of these are put in place to mitigate or manage identified risks, while others fall under the category of stewardship.

In the context of GM crops, stewardship refers to management and handling practices that are put in place to ensure the efficacy of the technology, seed purity, and intellectual property rights. Also, there are some programs that aim to ensure that certain grains are not mixed with others, as they have been modified for a particular type of processing, or they cannot enter certain markets as they have no import approval. The purpose of stewardship is **not** to ensure environmental safety. Measures to ensure environmental safety fall under the category of risk management.

At common requirement from authorities is to establish a monitoring programme to ensure that farmers are aware of how to use Bt crop technology in a way that will delay the development of insect resistance. This includes the implementation of refuge areas within the crop.

Risk communication is another component of risk analysis that is essential at all phases of risk assessment and risk management. Risk communication supports the overall analysis by linking and providing feedback between components, as indicated by the arrows in Figure 11. It also delivers information transparently to the various stakeholders, ensuring they are aware of the findings from the risk assessment and the reasons for any risk management decisions that might get made (Hautea, 2009).

In addition, risk communication can convey information to risk assessors on hazards perceived by the public before and during risk assessment, and on observed harm or on new risks. It also brings the public perception to the attention of decision-makers after the ERA is completed, where it might influence the final decision. Finally, risk assessment can be reviewed and adjusted if scientific journals or official reports reveal new scientific information that shows new risks

or actual harm to the environment (Sensi et al., 2011).

Risk communication can be challenging, because different people perceive and react differently to any given risk. Even the way that one person will perceive a given risk will vary depending on various circumstances. Thus, risk is a dynamic factor that changes depending on the person and the place. Furthermore, risk communication must reach a variety of people, all of whom will differ in education, cultural values, and tolerance for any given risk (Teng et al., 2012). Therefore, to be effective, risk communication must include objective goals that are measurable. As long as these are present, it can be supplemented with less tangible goals, such as cultural considerations.

The debate is not about science alone, but encompasses many issues usually included in some part of the risk analysis. Effective communication requires that the risk assessment process be described in a non-emotional way, using language that is clear and easily understood by all. The reason is because risk communication involves not only risk assessors and risk managers, but also other interested parties from the public and private sectors, including consumers, government, industry, academia, and non-governmental organizations, who do not have the necessary background to understand highly technical communication.

Risk communication must always emphasize the scientific aspects of risk assessment as the core of risk analysis, and the experience of GMOs in several countries since their first commercial release in 1994. For example, it is always helpful to remember that the GMO that is assessed is the result of a selection process during which all events with unexpected or unwanted behaviors were eliminated, and selected events passed strict quality and safety criteria, as shown in Figure 10; These results get reviewed by numerous different regulatory agencies around the world. There is no specific reason to expect that the elite events selected for marketing will behave differently than predicted. In every case, communication should be based on reliable information with sound scientific support and in a language that is accessible to target audiences.

Risk communication is embodied in Article 23 of the Cartagena Protocol on Biosafety, which compels the Parties to "promote and facilitate public awareness, education, and participation" as related to LMO safety; to "consult the public in the decision-making process;" and inform them on "public access to the Biosafety Clearing House."

For further guidance in risk communication, the World Health Organization – Food safety risk communication, http://www.who.int/foodsafety/micro/riskcommunication/en/ .

Communication and labeling

Article 23 of the Cartagena Protocol requires Parties to promote and facilitate awareness, education and the participation of the public on topics covered in the Protocol concerning GMO crops. However, the Protocol does not require Parties to label GMO crops as part of a communication strategy. In fact, compulsory labeling has the potential to conflict with Technical Barriers to Trade (TBT) of the World Trade Organization (WTO) Agreements. The Parties to the TBT Agreement are required to treat "similar products" equally to avoid discrimination against "similar products." Mandatory labels on various products (e.g., country of origin labeling, forest protection labeling, etc.) have given rise to several important disputes in the WTO and to resolutions unfavorable to laws and regulations. For example, see the WTO ruling in the Canada/Mexico vs. U.S. (country of origin labeling case) and Mexico vs. the United States of America (tuna-dolphin case, over labels that claimed that canned tuna was caught without harming dolphins).

Chapter 5: Regulatory Scenario

Biosafety is regulated in many countries by a set of specific laws, regulations, agreements, or policies. For these to be functional, these must be based on sound, authoritative scientific principles, and not on speculative or improbable risks. Caution is exercised through risk assessment as a predictive element of expected future behavior, which leads to decision making. Proper interpretation of the ERA should ensure environmental and food safety without unnecessarily compromising technological progress.

In a scenario with clearly assigned responsibilities, confined releases and laboratory and greenhouse experiments tend to be allowed without much delay, since these usually produce the data needed to assess safety in the country seeking to use them. For example, Argentina, Brazil, Colombia and Honduras have achieved great success in adopting GM crops, even though their regulatory frameworks are very different. There are four points in common among the three countries:

a environmental risk assessment only considers the direct biological impact of the GMO on the environment, food and feed safety assessments are done separately.

b social and economic aspects inherent to product adoption are analyzed separately from the risk assessment;

c the final decision for the commercial release is mainly based on the risk assessment recommendations;

d if the social and economic consequences of adopting a GMO are considered to be harmful to the country, commercial release is revoked or denied, according to the decision-making process in the country.

Each country should follow a clear procedure to authorize the commercial use of a GMO, which should be explicit in its legal framework. The criteria for a successful regulatory framework (modified from ACRE-DEFRA, 2007) are listed below, indicating for each if it belongs to risk assessment or to other parts of risk analysis:

- Consider both benefits and risks (analysis)
- Be scientifically-based (analysis and assessment)
- Might require small-scale trials to assess impact before broad use (assessment)
- Compare the new crop technology with current crops and practices, and not to some theoretical standard (analysis and assessment)
- Protect innovative opportunities and avoid dismissing biotechnology in favor of more harmful crops and practices (analysis)
- Be easy to implement (analysis)
- Consider the competitiveness of the agricultural sector (analysis)
- Consider the cost of inaction and maintaining the *status quo* (analysis)

A comparison between these criteria and the four points listed previously that characterize regulatory systems in place in GMO product-adopting countries show the importance of separating risk assessment from other considerations, including management and communication, which, as stated above, constitute risk analysis.

The importance of a properly drafted regulatory framework cannot be underestimated. The Brazil case is useful to illustrate the issue as described in Appendix I.

PART II: A Case Study

1. Golden mosaic virus-resistant Embrapa 5.1 bean (EMB-PV051-1)

This case study shows how the ERA for a GM bean was conducted. Event EMB-PV051-1 (hereafter referred to as Embrapa 5.1) was developed in Brazil by the Brazilian Company for Agriculture Research (Embrapa) to control the Bean Golden Mosaic Virus (BGMV), a devastating disease in common bean crops in Brazil. In contrast with the two previous case studies, this example shows how the ERA was conducted for a product that had not been commercialized in other countries. Also, in contrast with the two previous case studies, this GM crop does not express any novel proteins, it uses an RNA interference (RNAi) strategy that results in beans highly resistant to BGMV.

Figure 16: Embrapa 5.1 Bean Golden Mosaic Virus-resistant - BGMV (left), without symptoms.

1.1 Problem formulation - Defining the context

The main elements considered, while setting the context for the ERA of this product, where the legal framework, the biology of conventional beans, the gene construct used, the inserted elements, the use proposed and the receiving environment.

a. The legal framework

A detailed description of the Brazilian regulatory framework is shown in example 5.1, sub-item 5.1.1.a.

The specific protection goals considered were similar to those used for the previous two examples: water and soil quality, valued non-target organisms and agroecosystems. The scope considered areas where this GM bean could be planted and the neighboring areas.

b. The biology of the common bean

The common bean (*Phaseolus vulgaris* L.) originated in the new world (Vavilov, 1951) and was independently domesticated in Mesoamerica and in the Andes of South America (Gepts, 1998; Gepts et al, 2008; McClean et al, 2008; Kwak et al, 2009; Aragão, 2011). A center of secondary diversity is in the mountains of Peru and it appears that there could be an additional center of domestication in Colombia. Morphological evidence indicates that the wild bean that gave origin to the common bean is widely distributed in the United States, Western Mexico and the northeast region of Argentina, but is not present in Brazilian territory. The germplasm of cultivated beans can be

divided into a variable number of lines according to two systems proposed by Evans (1973) and Singh (1989). The wild type, can produce viable hybrids with the cultivated forms of *Phaseolus vulgaris*, and therefore is considered to belong to the same biological species.

The domestication of beans resulted in plants with more compact, erect growth, showing a marked enlargement of vegetative parts that increase the size of pods and seeds. In addition, there was loss of sensitivity to photoperiod and latency in the seed, and the reduction of pod dehiscence (Smartt, 1978, 1980).

All species of the genus *Phaseolus*, are diploid (2n = 22). This includes many species, of which only four are cultivated: *P. vulgaris, P. coccineus, P. acutifolius Gray var. Freem* and *P.s latifiolius lunatus var. lunatus* (Evans, 1976). All these species are likely to have arised from a common ancestor;

- *P. vulgaris* was domesticated in temperate zones of either Central or South America, or both (10,000 to 7,000 Before the Common Era (BCE));
- *P. acutifolius* was domesticated in semi-arid regions of Central America;
- *P. lunatus* was domesticated in South or Central America, or bothand is subtropical (4,500 BCE. in South America and 1,800 BCE. in Central America).
- *P. coccineus*, the only one whose reproduction is regularly achieved by cross-pollination (allogamous) was domesticated in the cold and mountainous areas of the Andes (2,000 BCE).

Of the four cultivated species, *P. vulgaris* (common bean) is the most important for human consumption.

Growth habit is an important morphological feature that has a direct influence on bean crop management. Growth habit can be determinate or undeterminate. Determinate growth is common to the cultivated species of *Phaseolus*, and it is characterized by the full development of the terminal meristem in an inflorescence, which is a trait controlled by a recessive gene.

There are both annual and perennial forms of *Phaseolus*. Annual forms are common in *P. vulgaris* and *P. acutifoflus*. Under field conditions, its growth cycle ends with the senescence of leaves and the maturation of the pod. Perennial forms are very common in *P. lunatus* and *P. coccineus*. Flowering, yield, and pod ripening is continuous, while the process is relatively short.

Although beans are predominantly autogamous because of their floral morphology, several species of bees can potentially carry pollen and fertilize plants over a short distance from the source of pollen. However, the effective pollination of bees and other insects in commercial bean fields appears to be minimal.

One of the most devastating diseases in common bean production is the golden mosaic disease, caused by the Bean Golden Mosaic Virus (BGMV). BGMV is a virus that is transmitted by the whitefly, *Bemisia tabaci*, and is present in all the regions of the American continent where beans are grown. Infection with BGMV can cause losses from 40 to 100% in production, the levels of infestation depend on the incidence of the virus in the area, the time of bean cultivation and the bean cultivar. The search for cultivars resistant to BGMV started in the 1970s, but only varieties with low levels of tolerance were found. Currently there are no conventional bean varieties with an adequate level of tolerance to BGMV. There is tolerance in cultivars of Mesoamerican origin, especially against the closely related virus, the *Bean Golden Yellow Mosaic Virus* (BGYMV), but no immunity has been found. Given the lack of tolerant varieties, disease management of golden mosaic depends on chemical control methods that control the insect vector (whitefly). There are several efficient active ingredients available to control some whitefly adults, but resistance to the insecticide has been observed as a result of their continued use. In addition, the insecticide is efficient in controlling whitefly adults, but is unable to prevent virus transmission. Furthermore, the continuous application of insecticides increases production costs and does not discriminate between whiteflies and non-target organisms

c. The receiving environment

The proposed use of the Embrapa 5.1 bean considered in this ERA was the commercial cultivation in Brazil. Thus, the potential scale and production areas of current common bean production in Brazil were identified. In addition, valued non-target organisms that could be at risk in these areas were considered.

Brazil is the second largest bean producer in the world. The production of grains in 2010 was 3.16 million tons (FAO, 2012). The cultivation of this leguminous crop occurs in three different seasons of the year, in an area of approximately 3.42 million hectares (IBGE, 2010). The five major producing bean areas in Brazil are Paraná, Minas Gerais, Bahia, São Paulo and Goiás, which between 2003 and 2005 accounted for 67.4% of the national production (Figure 12). However, commercial cultivation also occurs in other areas of Brazil.

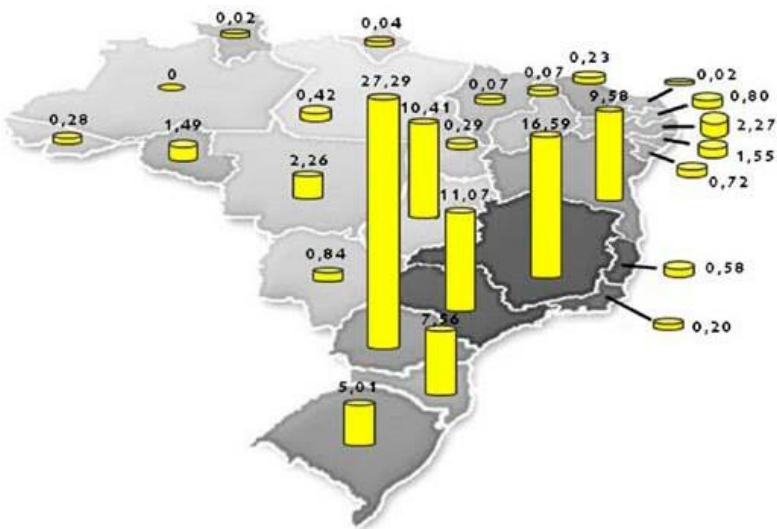

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Figure 17: Bean production in different Brazilian provinces for the 2003-2005 period. The height of vertical bars is proportional to the percentage of beans produced in each province.[2]

[2] Adapted from Technical Communication 187, Figura e, http://www.infoteca.cnptia.embrapa.br/bitstream/doc/857164/1/comt187.pdf

Over the past 20 years, the cultivation of beans in Brazil has experienced enormous changes, especially increased productivity, which is very marked in the third annual harvest, and the concentration of production in more developed regions: (a) to the south of Paraná and São Paulo, (b) in all the Federal Districts, and (c) in Bahía.

The bulk of bean production in Brazil in recent years, however, was not enough to meet domestic demand. Between 1998 and 2008, imports were approximately 100,000 tonnes a year, and the percentage of contribution from beans to the national requirements has remained stable. Official data show an increase in internal consumption accompanied by higher production (CONAB, 2012).

As noted in the previous section (Bean biology), there are no native species of wild bean in Brazil that can cross-pollinate with cultivated beans. In addition, the country is neither a secondary center of origin or a diversification center of *Phaseolus* (Debouck, 1988). Beneficial insects, especially predators, are important in bean cultivation in Brazil as they control populations of *Bemisia tabaci*, although practices of integrated pest management are just beginning to be adopted among producers.

d. Gene construct

The Embrapa 5.1 bean event was obtained using biolistics, as described by Aragão et al. (1996). A chimeric gene that expresses an RNA that contains a pair of fragments of the *rep* (AC1) gene of the BGMV genome was inserted in both sense and antisense directions with an intron linker (Fig. 13). The RNA from this vector folds over on itself to form a hairpin of double stranded RNA (dsRNA) which is recognized by a molecular complex within the cell that generates small RNA fragments known as *small interfering or siRNA*. These fragments interfere with the expression of the *rep* gene in the virus (Table 5), which is necessary for viral replication. As a result of the lack of expression of the *rep* gene, viral replication is compromised, and plants become resistant to the viral infection, functionally recapitulating the virus resistance naturally present in many plants. The expression cassette for the RNA hairpin is *ΔAC1hpRNA* ('hp' for *hairpin* forming dsRNA).

Figure 18: Schematic map of the pBGMVRNAiAHAS insert containing the genetic elements described in Table 1. The solid arrows indicate the PCR primers used for plant monitoring[1]

Genetic and molecular analysis revealed that the transgene was inserted into a single locus of the nuclear genome and have remained stable for several generations of selfing, crosses and backcrosses to conventional (non-GM) commercial varieties. In this event, no functional sequences of the *E. coli bla* gene, which encodes the beta-lactamase enzyme that confers resistance to beta-lactam compounds, such as ampicillin and is used as a marker for bacterial selection, but which was removed from the cassette prior to transformation.

GENETIC ELEMENT*	**FUNCTION**
P- ahas3'	Promoter and sequence leader of the *ahas* gene of *Arabidopsis thaliana (AtAhas)*
CS - ahas-cds	Coding sequence of the AHAS product (aceto-hydroxy acid synthase) of *A. thaliana* that confers resistance to herbicide imazapyr
T- ahas5'	Terminator of the *Atahas* gene
P-35S	35S promoter of the Cauliflower Mosaic Virus (CaMV)
AC1 (internal fragment)	Fragment of the viral replicase *AC1*) gene of the Bean Golden Mosaic Virus involved in viral replication
I – pdk	*pdk* gene intron of *Flaveria trinervia*
ocs3'	Terminator of the octopine synthase gene of *Agrobacterium tumefaciens*

ΔAC1	Genetic cassette within the insertion of the Embrapa 5.1 bean genome, which includes two copies of the 424-bp fragment of the BGMV AC1 interference and the 35S, pdk and ocs3 fragments

P – Promoter; I – Intron; CS – Coding Sequence ; T – 3' non-translated sequence with signals for transcript termination and polyadenylation *cf. Fig13

Table 4. Summary of Genetic Elements in Embrapa 5.1 event

The *AtAhas* gene was used as a selectable marker as it confers tolerance to herbicides of the imidazolinone class. The use of this gene permitted the selection of cell lines containing the transgene, but Embrapa 5.1 bean plants do not have a useful level of tolerance to this herbicide, thus, the event cannot be used as an alternative technology for weed control.

Small interference RNAs (siRNAs) encoded by the inserted transgene were detected in transgenic bean leaves but only trace levels were detected in fresh seeds. No siRNA were detected in cooked seeds. Further tests using bioinformatics did not show any unintentional silencing of genes from other leguminous plants, humans or animals.

e. History of the safe use of the event

This was the first time that an application for the commercial release of a virus-resistant GM bean event was presented to a regulatory body. Therefore, strictly speaking, there was no history of safe use of the GM product and no previous ERAs to refer to. However, other virus-resistant transgenic plants, such as papaya and squash, that also use the RNAi strategy, have been previously commercialized with no reports of adverse environmental effects.

1.2 Problem formulation - hazard identification and characterization

Once the context was established, then all potential risks associated with the commercial cultivation of this GM bean were considered.

Information provided by the developer on comparative assessments were taken into account to identify any biologically relevant differences between the Embrapa 5.1 bean and conventional beans. This included agronomic and phenotypic comparisons as well as comparisons of composition of key components.

The agronomic and phenotypic comparison of Embrapa 5.1 with conventional beans of the same cultivar in three regions of Brazil for two years showed that there were no biologically relevant differences in phenotype between these beans. Embrapa 5.1 beans do not produce new proteins that are different from conventional common beans. However, the siRNAs generated were considered a potential hazard as they could be ingested by non-target organisms and could potentially suppress the expression certain genes using the endogenous mechanisms of RNA interference.

The following potential risks were considered:

a) Adverse consequences of gene flow from Embrapa 5.1 beans to sexually compatible wild beans.
b) Adverse consequences of gene flow from Embrapa 5.1 beans to non sexually compatible species (horizontal gene transfer).
c) Increase in persistence or invasiveness of Embrapa 5.1 beans in comparison with conventional common beans.
d) Adverse effects on valued non-target organisms in comparison with conventional common beans.
e) Adverse effects Embrapa 5.1 beans on specific nutrient absorption processes by beans.
f) Adverse effects of Embrapa 5.1 bean cultivation on soil organisms or soil function in comparison with conventional common beans.
g) Potential changes in the sequence of the BGMV induced by the Embrapa 5.1 bean defnse mechanism, could lead to the rise of a “super virus”

1.3 Problem formulation- Exposure characterization

Once the specific protection goals and assessment endpoints to consider in the ERA were defined and the hazards identified, an exposure characterization was conducted. This included:

- mapping common bean producing areas in Brazil where Embrapa 5.1 could be cultivated,
- mapping where plant species sexually compatible with common beans are found
- identifying valued non-target organisms associated with conventional common beans and their potential exposure.
- mapping bean tissues that produce siRNAs from the introduced RNA.

1.4 Risk characterization

As described in Chapter 1, risk is a function of hazard and exposure, so taking into consideration the hazards identified, their potential impact on the assessment endpoints selected and potential exposure, a risk characterization was conducted.

Below is a summary of the approaches taken to assess the risk of cultivating Embrapa 5.1 bean in Brazil considering the risks listed earlier.

a) Adverse consequences of gene flow from Embrapa 5.1 beans to wild beans.

The information gathered in the context step of problem formulation and the exposure characterization showed that there are no sexually compatible wild relatives of the common bean in Brazil. Thus the risk that gene flow to these plant species would result in adverse environmental effects is negligible (no exposure).

b) Adverse consequences of gene flow from Embrapa 5.1 beans to non-sexually compatible species (horizontal gene transfer).

As in the previous case study, the potential adverse effects that horizontal gene transfer from the Embrapa 5.1 bean to other species was considered. Data from many scientific publications show that the horizontal gene transfer between unrelated species is a rare event (Keese, 2008). In this case, Embrapa 5.1 beans do not contain any antibiotic resistance markers, they do not produce any novel proteins and the RNA produced is highly specific for recognition by the BGMV and mEPSPS, and is unlikely to confer any selective advantage. This information combined with information from many scientific publications (see Keese, 2008 for a review) provided a weight of evidence that indicate that horizontal gene transfer would be a rare event and if it did happen it would have very minor consequences. The risk was therefore considered negligible.

c) Increase in persistence or invasiveness of Embrapa 5.1 beans in comparison with conventional common beans.

The risk that the genetic modification in Embrapa 5.1 beans could have resulted in any phenotypic changes that could lead to an increase on persistence or invasiveness of these bean plants, in comparison with conventional common beans, was assessed. The concern was that an increase in persistence could lead to agronomic problems due to an increase of volunteer bean plants in subsequent crops. Also, that an increase in invasiveness could result in Embrapa 5.1 bean plants establishing wild feral populations outside bean fields.

For this assessment, the phenotypic comparison data provided by the developer was examined. These data showed that there were no biologically significant differences in phenotype between Embrapa 5.1 beans and conventional common beans apart from the tolerance to BGMV.

The information on common bean biology compiled during the context step showed that conventional beans do not establish feral populations outside crop fields. Thus these characteristics are unchanged in Embrapa 5.1 beans. Tolerance to BGMV in the GM crop could be considered a selective

advantage, however this would only be the case in situations where BGMV was the limiting factor stopping the spread of bean plants in areas outside crops, which is not the case, as there are many other factors preventing their spread. The conclusion was that Embrapa 5.1 beans will not be more persistent or invasive than conventional common beans.

d) Adverse effects on valued non-target organisms in comparison with conventional common beans.

Another specific protection goal considered in the ERA was the potential adverse effect that cultivation of Embrapa 5.1 beans could have on valued non-target organisms. Considering the information from the comparative assessment, the only biologically significant difference in phenotype between Embrapa 5.1 beans and conventional common beans is the tolerance to BGMV. As discussed in the previous sections, Embrapa 5.1 beans do not produce new proteins that are different from conventional common beans, but one of the hazards identified were the siRNAs generated by the internal dicer enzyme in beans. The concern was that those siRNAs could potentially suppress the expression of important genes of valued non-target organisms.

Information regarding the mechanism of RNA interference shows that this is only active on eukaryotes, thus prokaryotes would not be at risk. The ERA then focused on two groups of valued non-target organisms considered important in bean crops: thrips (as predators of *Bemisia tabaci*) and mycorrhizae (as important organisms for the nitrogen fixation in beans). In order assess the potential risk to these organisms, the pathway to harm approach was used.

For the suppression of endogenous genes in non-target organisms to occur, first the insects would have to be exposed to bean tissues producing the siRNAs (either by feeding directly on plant tissues or indirectly through prey that fed on those tissues). Then complementarity between one of the siRNA generated inside the bean cells (a product of the excision of the double-strand) and endogenous mRNA of the non-target organism would be required. If recognition occurred, this would have to lead to the suppression of the expression of important insect genes. This suppression would have to be long lasting and result in important physiological changes in a significant

portion of the non-target organisms exposed, to adversely affect entire populations.

Information found in the scientific literature suggests that the transfer of siRNAs from one organism to another, and specifically from one cell to another of different organisms, is unlikely (Ref?). The interaction between these siRNAs with endogenous mRNAs of the non-target organism is also highly unlikely. Thus, the information available suggested that cultivation of Embrapa 5.1 beans is unlikely to result in adverse effects on valued non-target organisms.

e) Adverse effects of Embrapa 5.1 bean cultivation on soil organisms and soil processes in comparison with common beans.

To assess whether Embrapa 5.1 bean cultivation will result in adverse effects on soil organisms or processes in comparison with conventional beans, the results of the agronomic and phenotypic comparison were taken into account. No biologically relevant differences in phenotype were observed between Embrapa 5.1 beans and conventional common beans apart from tolerance to BGMV provided by the production of the double strand of RNA. Since the mechanism for RNA interference only acts on eukaryotes, soil bacteria and other prokaryotes are not at risk. As discussed in the previous section, no adverse effects on fungi are expected. Agronomic and compositional comparisons showed that Embrapa 5.1 beans and conventional common beans grown side-by-side did not show any biologically relevant differences. The conclusion was that the risk to soil microorganisms and soil processes can be considered negligible.

f) Potential changes in the sequence of the BGMV induced by the siRNA expressed by Embrapa 5.1 or by recombination of the elements of the genetic construct that would lead to the rise of a "super virus".

One of the concerns considered was whether the cultivation of Embrapa 5.1 could lead to changes in the BGMV resulting in a more viral strain of this virus. In order to assess this, information on the biology of BGMV virus was considered.

The BGMV virus replicates in the nucleus of the bean plant cells, thus recombination with regions of high homology in the viral sequence would be theoretically possible. However, only the part of the genetic construct inserted in Embrapa 5.1 beans that determines the generation of double-stranded RNA has homology with the virus. Changes in the original viral sequence that is part of the insert would be detrimental to the virus. Therefore, the probability that a competitive advantage would arise is very low. The probability of recombination between sequences added to the chromosome of the plant cell and viral DNA is very low. In addition, there is no advantage for the virus to change the sequence of the original *rep* gene for another against which the RNA interference mechanism is directed. As a result it can be concluded that the damage from this change will be dismissed.

1.4 Risk estimation

Once the risk characterization step was conducted, the conclusion was that cultivation of Embrapa 5.1 beans in Brazil will be as safe for the environment as cultivation of conventional common beans. The risk was estimated for each of the specific protection goals using the criteria outlined in Chapter 1. The likelihood of harm to sexually compatible wild relatives, to valued non-target organisms and to soil organisms or soil processes following the cultivation of Embrapa 5.1 beans was estimated as “highly unlikely” and the consequences “marginal” or “minor”, resulting in a risk estimate of “negligible” for all sections.

		RISK ESTIMATE			
PROBABILITY	Very high	Low	Moderate	High	High
	High	Low	Low	Moderate	High
	Low	Negligible	Low	Moderate	Moderate
	Very low	Negligible	Negligible	Low	Moderate
		Marginal	Minor	Intermediate	Major
		CONSEQUENCE			

Table 5. Estimation of risk of Embrapa 5.1 bean cultivation in Brazil to populations of wild relatives, non-target organisms and soil processes.

1.5 Recommendation

After following the process described above, the recommendation was to approve the commercial release of Embrapa 5.1beans in Brazil as they would provide an important tool to combat infestations that cause important yield losses of BGMV.

Appendix I

Significance of the regulatory framework

In Brazil, legislation is specific to the biosafety of genetically modified organisms and based on law No. 11105 of March 24, 2005, on its corresponding decree and in a series of policy resolutions regulating specific aspects of biosafety of GMOs, (http:www.ctnbio.gov.brindex.phpcontentview12840.html).

After substantial reform to the regulatory framework, the use of GMO crops has gained momentum since 2005, with more than 30 varieties of maize, cotton and soybean that are insect-resistant or herbicide-tolerant, and virus-resistant beans already approved for marketing (Figure 23). As a result, the adoption of biotechnology in the field has grown rapidly, with more than 90% of soy and 70% of corn being GMO (by 2011). In addition to GMO crops, Brazil has adopted the use of GMOs in vaccines and diagnostic tests, and in the production of enzymes, hormones, and fuel oil. By next year, it is expected that GMO animals will also be approved for commercial use, both in the production of food (pork, fish) and in the control of endemic diseases (mosquitoes). The adoption of new GMO crops, such as eucalyptus, sugarcane, and food crops important in Brazil, such as rice, is also anticipated.

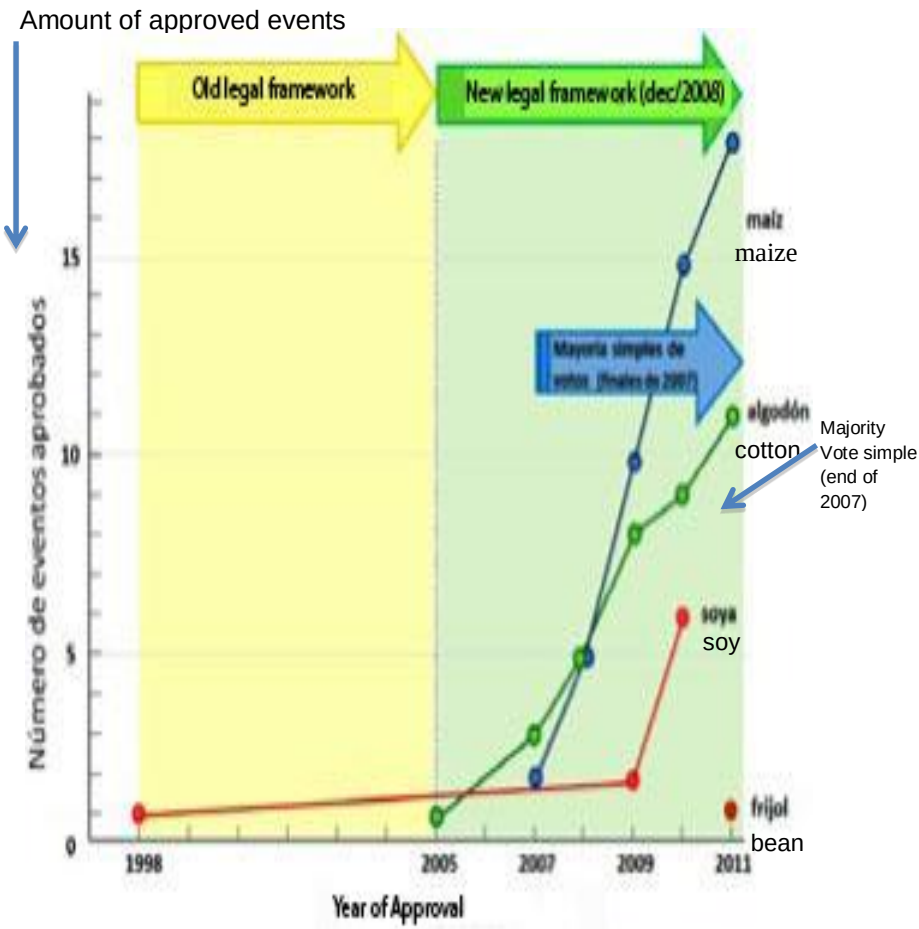

Figure 19: Number of events of genetically modified plants approved by CTNBio for commercial release in Brazil. There has been an initial increase in the number of varieties authorized since the passing of the new biosafety law (end of 2005) and a spike in the rate of approval after adopting the criterion of accepting applications for commercial release with a simple majority of votes in the plenary (mid-2007). This also highlights the fact that corn was adopted more quickly than the other two crops.

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